Plasticity, Intraspecific Variability and Local Adaptation to Climatic Extreme Events of Ecotypes/Provenances of Key Plant Species

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1. Compendium

1.1. Short Summary of the Thesis

Climate change, and especially an increase of magnitude and frequency of climatic extreme events such as drought periods or heatwaves, will alter growing conditions for plants in the future. Persistent ecosystems, with long-living organisms, such as forest or permanent grassland will be particularly impacted by this development. The velocity of these changes is likely to occur at a pace, which species may not be able to keep track with by natural dispersal or genetic adaptation. Agriculture, forestry and ecosystem management must develop counteracting practices to secure the persistence and functioning of these ecosystems and thus their provision of goods and services. Therefore it is important to develop a better understanding how species and ecosystems may respond to future climatic stressors. Impact assessments, e.g. via climatic envelope modelling are prone to misinterpretations of the adaptive capacity of species, as they do not incorporate the intraspecific genetic and phenotypic differences that exist within the populations accross the distribution range of a species.

Yet, intraspecific variation may exhibit potential tools for the development of climate change adaptation strategies. Here, I focus on key ecosystems in Central Europe. In particular the selective use of plant provenances or ecotypes may help to make ecosystems climate-resilient without a potentially more problematic introduction of exotic species. Especially provenances from warmer, drought-prone regions, with a current climate similar to the projected one for Central Europe recently came into focus as potential substitutes for local provenances, as they might have developed local adaptations to climate conditions at their location of origin. Insights about the response of these provenances to changing averages and extreme event regimes are crucial for a reasonable use of within-species diversity in climate change adaptation.

First, the concept of assisted colonization or migration of species or ecotypes and the role it can play as an adaptation strategy in agriculture, forestry or nature conservation is introduced (Manuscript1). It is suggested that a focus should be laid on keystone species that ensure ecosystem persistence and functioning as they govern the habitat structure and microclimate of a site. The assisted colonization of pre-adapted ecotypes of keystone species from climates similar to future projections for the target site is proposed.

Furthermore, provenances of selected grassland and forest key-species were exposed to drought and warming in two experiments in Bayreuth and Landau, and their ecological responses were analysed. Results suggest that local adaptations to climatic stressors exist. However, the magnitude and direction of responses strongly depend on species and climatic variables. For grassland species, e.g. differences in drought sensitivity could be demonstrated in some cases (Manuscript 4). *Fagus sylvatica* exhibited differences between the provenances in response to drought conditions, as well (Manuscript 3). It seems that marginal provenances, from the dry margins of the distribution range, show less increment reduction due to the drought treatment. Yet, under more favourable conditions of water supply these provenances did not yield the same high increment rates than more central provenances, indicating a trade-off between stability under stress and yield under non-stress conditions. A pine species that is generally considered to be rather drought-resistant, *Pinus nigra*, which is a potential substitute for climate-threatened conifers on dry sites in Central Europe, did not show any differences in response to drought and warming (Manuscript 2), maybe due to a weak selective pressure as a result of high drought-resistance across the whole distribution range. The impacts of drought on increment became not visible before the second year after the treatment, stressing the need for more long-time experiments in climate impact research.

Even in a generally warmer environment, cold extremes in winter or spring are expected still to prevail in the future. Therefore, the provenances of the selected species were tested for their cold-hardiness and late frost resistance (Manuscripts 5-7). Growth of the grassland species and *F. sylvatica* were negatively impacted by a late frost event and differences in late-frost sensitivity between provenances or ecotypes were identified. The (sub-) mediterranean species *P. nigra* showed differences between provenances in their winter cold hardiness. Correlations between performance under cold stress and winter conditions or late frost proneness of the places of origin could be established for almost all species. However, preceding climate experience, such as the warming or drought treatment of the plants altered their reaction to cold extremes compared to the control treatment, indicating the complexity of the interactive impacts of climate factors on ecosystem and plant performance.

The uncertainty of climate projections and the multitude of changing climatic stressors, though, make the prospect of an easy and rapid success in the search for single “best-adapted” provenances very questionable. In economics the *portfolio effect* shows that a diversification of investments decreases the risk of a total loss of profits. Hence, in a modelling procedure based on the increment data from the above mentioned experiment it was tested if a “portfolio

investment" in several provenances in one stand decreases the risk of yield losses (Manuscript 8). Results indicate that the higher the number of provenances the higher the chance for a "best-performer" to be included in the set. So the likelihood of higher yields, under different climatic conditions increases, yet the risk of low yields stays stable.

Generally, it seems that the selective use of plant species and ecotypes in climate change adaptation can be a feasible tool to maintain ecosystem functionality and productivity. However, the uncertain projections, the multitude of climatic stressors and their interplay with other environmental factors and the potential impacts of assisted colonization of ecotypes on the genetic diversity within species and populations require further research.

1.2. Kurze Zusammenfassung der Doktorarbeit

Der Klimawandel, und vor allem Veränderungen in Auftretenswahrscheinlichkeit und Intensität von Extremereignissen, wie Dürren oder Hitzewellen, werden die Wuchsbedingungen für Pflanzen künftig stark ändern. Hochstete Ökosysteme, wie extensiv genutztes Grünland oder Wälder mit langlebigen Organismen werden besonders betroffen von diesen Veränderungen. Die Geschwindigkeit mit der sich dieser Wandel vollzieht, macht es für viele Arten schwer bis unmöglich durch Arealverschiebung oder genetische Anpassung Schritt zuhalten.

Vor diesem Hintergrund muss die Land- und Forstwirtschaft Gegenmaßnahmen entwickeln, die die Funktion dieser Ökosysteme erhalten und die Erträge und die Bereitstellung von ökosystemaren Dienstleistungen sichern. Das Wissen um die Auswirkungen klimatischer Stressfaktoren auf Arten und Ökosysteme ist daher unerlässlich. Die Einschätzung solcher Auswirkungen, z.B. durch Klimahüllenmodellierung, berücksichtigt die innerartliche genetische Vielfalt und phänotypische Plastizität, die innerhalb und zwischen Populationen existiert, in der Regel nicht, was zu Fehleinschätzungen der Anpassungsfähigkeit führen kann.

Diese innerartliche Variation kann jedoch ein wichtiges Werkzeug darstellen, wenn es um die Klimaanpassung von Ökosystemen in Mitteleuropa geht. Dieser Studie konzentriert sich auf Schlüsselökosysteme in Mitteleuropa. Die selektive Nutzung von Herkünften oder Ökotypen wichtiger Schlüsselarten kann eventuell dazu beitragen Ökosysteme resilienter gegenüber negativen Klimaeinflüssen zu machen, ohne dabei die oft problematische Einführung von exotischen Pflanzenarten in Kauf nehmen zu müssen. Dabei geraten besonders Herkünfte aus wärmeren und trockeneren Gegenden, mit Klimabedingungen ähnlich zu den für Mitteleuropa

prognostizierten in den Fokus, da diese eher solche Bedingungen angepasst sein könnten. Es ist jedoch wichtig zu wissen, wie verschieden Herkünfte auf sich verändernde Mittelwerte und Klimaextreme reagieren, um dieses Mittel vernünftig einsetzen zu können.

In der vorliegenden Arbeit wird das Konzept der *Assisted Colonization* vorgestellt und beschrieben welche Rolle es in Land- und Forstwirtschaft und im Naturschutz spielen könnte (Manuskript 1). Es wird dargelegt. Dass dabei vor allem Schlüsselarten im Fokus stehen sollten, da sie oftmals Habitatstrukturen und Mikroklima und damit auch Bestand und Funktion von Ökosystemen bestimmen. Die gezielte Einfuhr von angepassten Ökotypen aus Regionen in denen heute Klimabedingungen herrschen, wie sie für die Zielregionen prognostiziert werden, wird hierbei zu Diskussion gestellt.

Weiterhin wurden Herkünfte ausgewählter Grünland- und Baumarten in einem Topfexperiment künstlicher Dürre und Erwärmung ausgesetzt, um deren Reaktion darauf zu messen. Die Ergebnisse zeigen, dass es lokale Anpassungen an klimatische Stressfaktoren gibt, diese jedoch artspezifisch variieren und stark von der jeweiligen Klimavariable abhängen. Unterschiede in der Dürreresistenz zwischen verschiedenen Herkünften mancher Grasarten (Manuskript 4), sowie zwischen Herkünften der Rot-Buche (Manuskript 3) konnten dabei nachgewiesen werden. Vor allem bei der Rot-Buche scheinen Herkünfte vom trockenen Rand des Verbreitungsgebietes weniger stark auf Dürre zu reagieren wie Herkünfte aus dem Zentrum des Verbreitungsgebietes. Jedoch konnten diese „Rand-Herkünfte“ unter günstigen, ausreichend wasserversorgten Bedingungen auch nicht so hohe Ertragsleistungen erzielen. Hier geht Stabilität unter Stressbedingungen anscheinend auf Kosten hoher Erträge unter günstigen Bedingungen. Herkünfte der Schwarz-Kiefer, einer generell dürreangepassten Art, die als möglicher Ersatz für gefährdete Nadelbaumarten auf Trockenstandorten in Mitteleuropa gilt, unterschieden sich nicht in ihrer Reaktion auf Trockenheit und Erwärmung (Mauskript 2), was durch einen schwachen Selektionsdruck aufgrund einer allgemeinen hohen Dürreresistent im gesamten Verbreitungsgebiet erklärt werden könnte. Die Zuwachsleistung reagierte jedoch erst im zweiten Jahr auf das Dürreereignis. Dies unterstreicht die Notwendigkeit von langfristig angelegten Klimaexperimenten um die Auswirkungen von extremen richtig beurteilen zu können.

In einem weiteren Schritt, wurden die Herkünfte der genannten Arten auf ihre Frosthärte und Spätfrosttoleranz getestet, da solche Ereignisse auch in unter höheren Durchschnittstemperaturen dennoch möglich sein werden (Manuskripte 5-7). Spätfrostereignisse führten zu einem geringeren Wachstum bei den Grasarten als auch bei der

Rot-Buche und Unterschiede zwischen den Herkünften in der Spätfrosttoleranz konnten aufgezeigt werden. Auch die Herkünfte der (sub-)mediterran verbreiteten Schwarz-Kiefer unterschieden sich in ihrer Frosthärte. Die *Performance* unter Kältestress korrelierte bei fast allen Arten mit Minimumtemperaturen in Winter oder Frühling in den Herkunftsorten der verschiedenen Provenienzen. Interessanterweise beeinflussten die „Klimaerfahrungen" der Pflanzen, sprich die vorausgehenden Temperatur- und Dürremanipulationen, die Reaktion auf Frost, was die Komplexität des Zusammenspiels von verschiedenen Klimafaktoren und deren Auswirkung auf Arten und Ökosysteme deutlich macht.

Die Unsicherheit der Klimaprojektionen und die Vielzahl sich verändernder Klimafaktoren machen die Suche nach einzelnen bestangepassten Herkünften jedoch nicht sehr erfolgversprechend. In den Wirtschaftswissenschaften beschreibt der *Portfolio-Effekt* die Risikominimierung durch eine Streuung der Investitionen. In dieser Arbeit, wurde in einem Modell, basierend auf den Zuwachsdaten aus oben genannten Experiment, getestet, ob eine „Portfolio-Investition" in mehrere Herkünfte innerhalb eines Bestandes das Risiko von großen Zuwachsverlusten unter Stressbedingungen minimieren kann (Manuskript 8). Die Ergebnisse deuten darauf hin, dass mit steigender Anzahl von Herkünften die Chance steigt eine „Super-Herkunft" im „Portfolio" zu haben. Das bedeutet, dass die Chance auf hohe Erträge unter verschiedenen Klimabedingungen steigt mit steigender Zahl Herkünfte, das Risiko niedriger Erträge jedoch gleich bleibt.

Abschließend lässt sich sagen, dass die selektive Nutzung von Herkünften oder Ökotypen durchaus ein geeignetes Mittel zur Klimaanpassung sein kann. Die Unsicherheiten der Klimaprognosen, die Vielzahl klimatischer Stressfaktoren und deren Interaktion mit anderen Umweltfaktoren, sowie die Auswirkung von *Assisted Colonization* auf die genetische Vielfalt innerhalb von Populationen und Arten, macht jedoch weitere Forschung notwendig.

2. Background of the Thesis

2.1. Global Climate Change and Extreme Weather Events

Current anthropogenic climate change is unequivocal and is taking place at an unprecedented rate within at least the last 1,300 years (Brohan et al. 2006; Mann et al. 2008; Trenberth 2011a). Global mean surface temperatures have increased by 0.74°C (± 0.18°C) in the period 1901–2005, with a rate of temperature increase over the last 50 years that is almost double the rate of the last 100 years (Solomon et al. 2007). Warming has taken place both over land and ocean; however land surface temperatures have risen almost twice as fast as sea surface temperatures over the last 30 years, with the highest rates in Northern Hemispheric winter and spring (Solomon et al. 2007). In Germany the temperature increased about 0.8 – 1.0°C over the last century (1901-2000) (Zebisch 2005), which is above the global average (Beierkuhnlein and Foken 2008). There is a broad scientific consensus, that anthropogenic greenhouse gas emissions are the major driving force of the observed warming and that it cannot be explained by natural forcings alone (Solomon et al. 2007; Trenberth 2011a).

For the future, depending on the development of global CO_2 and other greenhouse gas emissions, an additional temperature rise between 1.1 and 6.4°C by the end of the century (2090-2099) compared to 1980-1999 is projected. The moderate A1B scenario (IPCC 2000), which is often used for climate impact assessments and corresponds to the 2°C target of the UN Climate Change Conference in Cancun, Mexico 2010, predicts a further warming of 2.8°C globally (Solomon et al. 2007) and an increase in annual mean temperatures of 2.3 to 5.3°C by the end of the century for Central and Northern Europe (Solomon et al. 2007) However, the actual CO_2 emissions increased by 29% from 2000 to 2008, which even exceeds the most pessimistic A1F-scenario (Le Quere et al. 2009; Smith et al. 2009).

Along with the rising mean surface temperatures, other climatic factors, like wind systems and precipitation patterns are expected to change, too. Changes in frequency and magnitude of extreme weather events may have a greater impact on ecosystems and society than changes in mean values (Easterling et al. 2000; Gutschick and BassiriRad 2003; Jentsch et al. 2007; Hegerl et al. 2011; IPCC 2012). New, unprecedented extremes may occur both through a shift of the mean of the distribution of a climatic variable or through changes in its standard deviation (Meehl et al. 2000). The future development of climatic variability and the connected trends in the probability of extreme events are harder to project than gradual changes (trends) in the mean of a climatic variable, however progress has been made in the observation and projection of these events (Solomon et al. 2007). Especially on the field of

temperature extremes the different climate models exhibit a relatively consistent picture, showing an increasing occurrence probability and duration of heat waves and a decrease in frequency, but not magnitude of cold extremes (Kodra et al. 2011; Orlowsky and Seneviratne 2012). The projections for changes in precipitation extremes (including droughts) seem to be more vague, even if some general trends towards prolonged drought periods over the Mediterranean and more heavy rain events in Northern high latitudes are emphasized (Orlowsky and Seneviratne 2012).

In the following, focus will be laid on observed and expected ecologically relevant changes in precipitation extremes, especially on changes in occurrence and duration of drought periods. Furthermore the development of temperature extremes will be illuminated with special attention on, often neglected, cold extremes on the background of a general warmer world.

2.1.1. Temperature extremes

For the 20th Century a lengthening of the frost-free season, a decreasing frequency of cold extremes and an increase in frequency of extreme high temperatures has been reported (Easterling et al. 2000; Salinger 2005; Solomon et al. 2007). In Europe, summer heat waves occurred more frequently, especially since the 1970s and less frequent cold extremes, a lower number of frost days and higher maximum temperatures in winter in the second half of the 20th Century could be detected (Schaer und Jendritzky 2004; Alexander et al. 2006; Moberg et al. 2006; Solomon et al. 2007; Della-Marta et al. 2007; Kuerbis et al. 2009; Cattiaux et al. 2011; Unkasevic and Tosic 2011; Orlowsky and Seneviratne 2012) Especially in the Mediterranean a pronounced summer time warming was found (Kioutsioukis et al. 2010; Bartolini et al. 2012).

This development will even intensify in the course of the 21st century, with an upward trend in mean values, but also with increasing temperature variability and more frequent extremes (Beniston et al. 2007; Kjellstrom et al. 2007; Solomon 2007; Fischer and Schaer 2009; Ballester et al. 2010; IPCC 2012).

Despite the general warming trend, cold extremes in winter or late frosts in spring are still likely to occur in the future, even if being less frequent. Rigby and Porporato (2008) show that the late frost risk in a future climate is as sensitive to changes in variance of temperature, as to changes in mean, which means that the frost risk will stay more or less constant. Despite the fact that there will be fewer frost days in the future, severe frost events are still expected to

occur (Woldendorp et al. 2008). The intensity and duration of cold extremes might prevail in a future warmer world, even at the end of the 21st Century (Kodra et al. 2011), but the fewer occasions of extremes might be more scattered in time (Jylhä et al. 2008).

2.1.2. Precipitation extremes

Higher air temperatures are connected with higher evaporation and a higher water holding capacity of the atmosphere (7% / +1°) and thus will intensify the hydrological cycle (Huntington 2006; Min et al. 2011; Trenberth 2011b). One consequence of this fact is the expected increase of extreme heavy rain events. On a global scale precipitation indices exhibit a trend to wetter conditions throughout the 20th century (Alexander et al. 2006). For Europe, summer precipitation has become more intense, but rain events are less frequent (Moberg et al. 2006; Kysely et al. 2011), while until now a seasonal increase in precipitation could only be detected for winter (Della-Marta et al. 2007). However, projected changes in precipitation extremes (including droughts) are more vague than for the temperature regimes (Solomon et al. 2007; Trenberth 2011b; Orlowsky and Seneviratne 2012). In general, a pattern with increasing precipitation over Northern mid- and high latitudes and decreasing rainfall over Southern Europe and the Mediterranean (Orlowsky and Seneviratne 2012) becomes visible.

As this study deals mainly with extreme drought events, heavy rain events will not be adressed in detail, but focus will be put on the observed and projected changes in drought frequency and intensity and its impacts, which this study mainly deals with. The assessment of droughts turned out to be complicated as the emergence of drought conditions depends not only on precipitation regimes, but also on evapotranspiration driven by radiation and temperature anomalies and soil properties (Orlowsky and Seneviratne 2012). However, Dai (2011) found a significant trend towards an increase in dry areas (+1.27% per decade) on a global scale for the time period 1900 to 2008, using various forms of the Palmer Drought Severity Index (PDSI). Very dry areas (with PDSI below -3.0) were shown to have doubled in extent, primarily in the tropics and subtropics, since the 1970s (Solomon et al. 2007; Trenberth 2011b). In Europe many regions have experienced drought periods since the mid 20th Century, with increasing summer temperatures being the main reason for the drying trend (Schaer and Jendritzky 2004; Briffa et al. 2009; López-Moreno et al. 2010). Models predict that this trend will continue in the course of the 21st century due to increasing summer temperatures and a decrease of summer precipitation up to 30 % in the Mediterranean (Beniston et al. 2007; Blenkinsop and Fowler 2007; Solomon et al. 2007; Rowell 2009;

Kysely et al. 2011; Orlowsky and Seneviratne 2012), again especially for Southern and South-Eastern Europe and the Mediterranean region. However, also parts of Germany and Bavaria are likely to experience prolonged phases of dry conditions in the future (Schönwiese et al. 2005; Beierkuhnlein and Foken 2008).

2.2. Climate Impact on Ecosystem and Selected Species

2.2.1. General Climate Impacts on Ecosystems

The above mentioned recent climatic changes of mean values and frequency and magnitude of extreme events already triggers ecological responses, at all hierarchical levels, from single individuals to species, communities and ecosystems (Walther et al. 2002). Extinction risk is likely to increase by approximately 20 to 30% for plant species known to science, if the 2°C target will not be met, and adverse impacts on unique or threatened ecosystems will amplify (Smith et al. 2009).

Anthropogenic climate change through carbon emission may have positive stimulating effects on various ecosystem processes and functions. Higher temperatures and the accompanying extension of the growing period , increased solar radiation due to decreased cloud covers, CO_2-fertilization and CO_2-induced water savings due to reduced stomatal conductance may have positive impacts on net primary productivity (NPP) in some ecosystems (Leemans et al. 2002; Nemani et al. 2003; Morgan et al. 2004). However, vegetation response to climate change does not only have implications for individuals, communities and ecosystem functioning and services, but may impose feedbacks to the climate system. The reaction of large biomes, like boreal forests or the Amazonian rain forests may depict potential tipping points for the climate system (Lenton et al. 2008). Terrestrial ecosystems are likely to turn from carbon sinks into carbon sources by the middle of the 21st Century (Solomon et al. 2007).

Furthermore species composition of ecosystems are expected to shift due to extinction, invasion, changing dominance patterns, range size reductions or expansions, poleward or altitudinal range shifts, as species do not respond synchronously to climatic stressors (Bakkenes et al. 2002; Parmesan and Yohe 2003; Walther et al. 2005; Walther et al. 2009; Kardol et al. 2010; Murphy et al. 2010; Alkemade et al. 2011).

Climatic extreme events, however, may have stronger and more unpredictable impacts on ecosystems than gradual changes in mean values (Easterling et al. 2000; Jentsch and

Beierkuhnlein 2008; Jentsch and Beierkuhnlein 2010; Beierkuhnlein and Foken 2008; Smith 2011). The velocity of changes, especially an increased frequency and magnitude of extremes may exceed the ability of species and ecosystems to adapt in a timely manner in terms of natural dispersal or evolutionary adaptation processes (Thomas et al. 2004; Lindner et al. 2010; Chen et al. 2011; Zhu et al. 2012).

In the following the specific climate impacts on grassland and forest ecosystems will be presented, with a special focus on the key species adressed in this study.

2.2.2. Climate Impacts on Permanent Grassland Ecosystems

Permanent grasslands and hay meadows are an important part of European agriculture and occupy a large proportion of the landscape (13.2% of the total area or 568 042 km² within the EU 27; EUROSTAT 2010) and are therefore of high ecological and economic importance. A general warming trend, i.e. an increase in mean temperatures will most likely result in increased biomass production in most grassland ecosystems, yet this effect will be larger in colder regions where growth seems to be temperature limited (Rustad et al. 2001; Penuelas et al. 2004). However, de Boeck et al. (2007) found a negative impact on above and below ground productivity of experimental warming on grassland species due to warming-induced increase in evapotranspiration. Extreme heatwaves will have most likely negative impacts on grassland productivity, yet the timing of these heatwaves is of importance, as shown by Craine et al. (2012). Like for most plant species, the growing season is prolonged and the phenological patterns are changed by warmer temperatures (Menzel et al. 2006).

On the other hand, late frost events can have serious impacts on grass species (Manuscript 7) and grassland communities (Gu et al. 2008). A precipitate onset of the growing season due to a general warming trend may increase the risk of late frost damage, as the timing of the frost events is not changing (Gu et al. 2008; Inouye 2008; Rigby and Porporato 2008; Woldendorp et al. 2008). Late frost damages increase when the plants are hit in a phase where they have already lost frost hardiness that protected them during winter time (Liu and Osborne 2008).

Extreme precipitation events, including droughts, however, seem to have a higher impact on grassland productivity than temperature changes. The 2003 drought over Central Europe caused strong fluctuations of biomass productions e.g. in French grasslands (Ciais et al. 2005). Several experimental studies demonstrated productivity declines for grasslands due to drought conditions (Morecroft et al. 2004; Weisshuhn et al. 2011; Signarbieux and Feller

2011, 2012; Craine et al. 2012). Contrastingly, Jentsch et al. (2011) found no decrease of ANPP due to an experimental drought, which is in accordance with the finding that in mesic grasslands drought often has no long-term effects on below- and above-ground productivity (Naudts et al. 2011). In the Mediterranean, more arid regions the mean stable area of grassland is expected to decrease (Alkemade et al. 2011), but it has to be considered that Mediterranean grasslands are composed mainly of annual plant species and some geophytes and can hardly be compared to grasslands in Central, Northern and Western Europe, where perennial and clonal species predominate. Heavy rainfall exhibited only minor effects on grassland productivity (Kreyling et al. 2008b).

Despite minor effects on productivity, drought was found to alter belowground processes, like soil respiration and microbial activity (Sowerby et al. 2008; Jentsch et al. 2011). Kreyling et al. (2008a), though, could not detect drought impact on soil enzyme activities or decomposition rates.

Jentsch et al. (2009) found altered plant phenological patterns as a consequence of drought and heavy precipitation events in a grassland experiment. Drought extended the flowering period, whereas heavy rain reduced it.

Furthermore it seems that more complex, species-richer communities are more resilient to extreme weather events, like drought (Gielen et al. 2007; Kreyling et al. 2008b; Signarbieux and Feller 2012). In experiments, which combined warming and drought conditions as single and combined factors, the artificial heating intensified the negative effects of drought on productivity and survival, whereas heating alone showed no significant effects (van Peer et al. 2004; de Boeck et al. 2011).

In general, the response of grassland ecosystem to climatic change and extreme events is a complex interplay of community composition and species richness, geographic location, timing of events etc. and is not yet understood satisfactorily.

2.2.3. Climate Impacts on Forest Ecosystems

Forest ecosystems are particularly threatened by climate change, as they consist of long-living organisms, trees, what makes it difficult to adapt to rapid changes in a timely manner (Thomas et al. 2004; Lindner et al. 2010). The response of forest ecosystems to increased temperature or changes in precipitation regimes strongly depends on the geographic or bioclimatic region. In temperate oceanic or boreal forests, the negative impacts on forest

productivity are expected to be less severe than in more continental or Mediterranean forest ecosystems, where growth is already water limited (Lindner et al. 2010). In these water limited regions, high temperatures and drought conditions will most likely reduce forest productivity and facilitate tree mortality (Allen et al. 2010).

In Western and Central Europe especially native conifer species will suffer under increased temperatures along with reduced precipitation in summer, and might be replaced by more competitive deciduous tree species (Maracchi et al. 2005; Kölling 2009).

On the other hand, warmer winter temperatures have been found to reduce the frost hardening of trees, especially in the continental regions of Europe with harsh frost events during the cold months (Hanninen 2006; Lindner et al. 2010). Furthermore trees are expected to become more vulnerable to late frost events in spring as warmer temperatures trigger earlier leaf flushing (Kramer et al. 2000).

Furthermore a warmer and drier environment may improve conditions for herbivore insects (Vanhanen et al. 2007; Westgarth-Smith et al. 2007) and forest pathogens (Desprez-Loustau et al. 2007), resulting in calamities and the large-scale breakdown of forest stands.

However, these impacts of climate change on forest ecosystems are strongly species-dependent according to the dominant key tree species and can even differ in severity within the distribution range of one single species (Hlasny et al. 2011) as site conditions, phenotypes, and regional climatic changes may differ (Lindner et al. 2010).

In the following observed on protected impacts of climate change and extreme events on the two tree species, used in this study, will be presented.

Fagus sylvatica

Fagus sylvatica is the naturally dominant tree species in Central Europe, and therefore of high economic importance. The area stocked with *F. sylvatica* constantly increases in Central Europe/Germany due to forest conversion from coniferous to mixed or deciduous stands, despite the fact that it is considered a “high-risk” species in terms of climate change (Rennenberg et al. 2004; Ohlemuller et al. 2006; Gessler et al. 2007; Scherrer et al. 2011). Particularly due to its drought-susceptibility it deserves special attention in the face of changing growing conditions in the future (Fotelli et al. 2009). The southern edge of the recent distribution range of *F. sylvatica* is most probably limited by drought events (Jump et al. 2006; Maxime and Hendrik 2011). In France, the observed recent decline in beech forest

productivity could be linked to decreasing water availability in early summer (Lebourgeois et al. 2005; Hewitt et al. 2011). Furthermore dendroclimatological studies in the Apennine Mountains in Italy revealed a strong correlation of basal area increment (BAI) of beech stands with water availability. Since the 1970s BAI has been declining due to water limitations (Piovesan et al. 2008). However, also in Central Europe drought events negatively impact the performance of beech. The exceptional drought period and heatwave over central Europe in 2003 generated distinct negative effects on the regeneration, growth, mortality and photosynthetic activity of F. *sylvatica* (Czajkowski et al. 2005; Leuzinger et al. 2005; Jung 2009; Betsch et al. 2011). Moreover, drought periods increase the pathogen- and fungi-susceptibility of European beech, as shown by Jung (2009) for the post-2003 years for a Bavarian *F.sylvatica* stand.

Figure 1: Drought damages in *Fagus sylvatica*, *Carpinus betulus* and *Quercus robur* during the 2003 drought close to Bayreuth (Bad Berneck). Photo: C. Beierkuhnlein

In the future, growth and distribution of *F. sylvatica* is expected to decline especially at lower elevations at the southern range limit (Matyas et al. 2009; Hlasny et al. 2011), but also on xeric sites in Central Europe (Czucz et al. 2011). Under drought conditions the regeneration of *F. sylvatica* will be threatened, especially in understorey due to its conservative shade-tolerant growth strategy (Robson et al. 2009). Furthermore it is observed and projected that *F. sylvatica* loses its competitive advantage to less drought-sensitive species, like *Quercus petraea*, under water limited conditions, especially on the Southern and South-Eastern distribution edges (Bonn 2000; Fotelli et al. 2001; Leuzinger et al. 2005; Friedrichs et al. 2009; Clark et al. 2011; Scharnweber et al. 2011), whereas Bolte et al. (2010) showed that on the Northern margin of the species' distribution range climate change may bring competitive advantages for *F. sylvatica* versus coniferous trees such as *Picea abies*.

One further aspect that can not be neglected is the fact that warmer winter and spring temperatures may will lead to earlier leaf flushing in *F. sylvatica* and thus increase the risk of late frost damage (Kramer et al. 2000, Kreyling et al. (2012).

Pinus nigra

Pinus nigra, with its (sub-) Mediterranean distribution is considered very drought-tolerant (Isajev 2004; Huber 2011), and was therefore identified as a potential substitute for conifer species, like *Picea abies* and *Pinus sylvestris* that are threatened by the changing climate in Central Europe. *P. nigra* exhibited a significantly lower mortality rate after two natural droughts than *P. sylvestris* in Eastern Spain (Martinez-Vilalta and Pinol 2002). Lebourgeois et al. (1998) showed that the drought tolerance of *P. nigra* saplings is attributed to their capacity to effectively counteract water stress by stomatal control of transpirational water loss. Yet, a trade-off between this survival strategy under water limited conditions, and a reduction in growth becomes evident. Especially, late-wood formation was found to be sensitive to the precipitation regime during summer months (Biel et al. 2004; Martin-Benito et al. 2008; Martin-Benito et al. 2010). In addition to an immediate growth response to drought conditions, delayed growth declines have been reported for *P. nigra*. Precipitation and temperature conditions of the previous year impact the growth, especially in the early phase of the growing season (Lebourgeois 2000; Andreu et al. 2007; Martin-Benito et al. 2008).

Dendrochronological data suggest that impacts of increasing temperature on the performance of *P. nigra* will differ between geographic regions (Martin-Benito et al. 2010). Warming stimulates growth in the more temperate parts of its distribution, while it increases water stress in the Mediterranean region and thus negatively effects the growth of this species.

2.3. Within-species Diversity as a Potential Tool in Climate Change Adaptation

Given the above mentioned velocity of climate change and the involved negative impacts on grassland and forest ecosystems, nature conservation, agriculture and silviculture have to adopt counteracting practices that aim on supporting dispersal and facilitating adaptability in order to maintain ecosystem functioning and thus the provision of ecosystem goods and services. Traditional assessments of climate change impact on species with climatic envelopes (Thomas et al. 2004; Thuiller et al. 2005; Kölling 2007) might misinterpret the adaptive capacity of a species to changing conditions. Within-species diversity is potentially important in this context.

Populations within species or taxa are known to differ phenotypically. Provenance-trials have a long tradition in forestry and have been conducted for more than a century now (e.g. vonWuehlisch et al. 1995) Evidence from these trials suggests that differentiation within

species is distinct, at least in forest trees. For grassland species, only a few experiments considered within-species variation (Fetcher and Shaver 1990; Ryser and Aeschlimann 1999). These differences in phenotypic expression could be underlined on a genotypic level by molecular methods for forest trees (Magri et al. 2006) and common grass species (Michalski et al. 2010). Generally this phenotypic and genetic variation is expressed in local adaptation to climate conditions or other abiotic factors such as soil type (e.g. Joshi et al. 2001; Hufford and Mazer 2003; Savolainen et al. 2007; Chen et al. 2010; Ofir and Kigel 2010).

Especially species with large distribution ranges that cover a broad range of climatic conditions, such as *F. sylvatica* are likely to display high levels of within-species variation and adaptation to local conditions. In forestry, the introduction of provenances or ecotypes from regions within the distribution range of the species with current climatic conditions similar to the projected conditions for the target area has therefore been suggested as one potential tool in climate change adaptation (Hemery 2008; Bolte et al. 2009; Bolte and Degen 2010). For grassland species this has been not yet discussed on a mentionable level, yet, in spite of the deficit in studies which examine local adaptation and its implication for climate adaptation in grass species, Macel et al. (2007) found evidence for a local adaptation to climatic factors in two ecotypes of *Holcus lanatus.*

F. sylvatica exhibits a high genetic diversity within in populations in Central Europe (Konnert 1995; Vornam et al. 2004). Looking at the distribution range on a continental scale the genetic differences between populations become more distinct (Comps et al. 1990; Magri et al. 2006). The genetic composition and diversity of populations determine their phenotypic plasticity and thus their adaptive capacity (Schaberg et al. 2008; Matyas et al. 2009), so differences in genetic configuration most likely display differences in adaptive capacities between populations. In several provenance trials, distinct responses of provenances of *F. sylvatica* to climatic stressors, such as drought, have been demonstrated (Schraml and Rennenberg 2000; Peuke et al. 2006; Czajkowski and Bolte 2006). Evidence for macroclimatic adaptation could be detected in a European-wide provenance-trial network, where the performance of different provenances was negatively correlated with climatic distance between test-site and origin of provenance (Matyas et al. 2009). Yet, also in the field local adaptations to drought are found. In the extraordinary dry year 2003, beech populations in Greece only experienced mild drought stress compared to Central European beech forests (Fotelli et al. 2009), which indicates an adaptation of Greek populations to drought conditions. Especially these marginal populations, which face more adverse conditions and are thus under stronger genetic selection

(Wortemann et al. 2011), are therefore under focus in the search of drought-resistant ecotypes (Rose et al. 2009).

Supported by its scattered distribution range, *P. nigra* also shows strong genetic differences between populations and subspecies (Jagielska et al. 2007; Soto et al. 2010). This genetic differentiation is supposed to have been enhanced by geographic isolation during the Pleistocene (Aguinagalde et al. 1997). Provenance trials showed a non-uniform performance of *P.nigra* provenances from various geographic origins (Varelides et al. 2001; Seho et al. 2010); however, differences in response to climatic stressors, such as drought, could not be proven yet, as the high drought tolerance across population might prevent a strong selection.

Considering the outlined potential impacts of changing climatic conditions on functions and services of grasslands and forest ecosystems it is important to know, whether specific provenances or ecotypes of key species are more or less susceptible or better adapted to climatic stressors, such as drought, heat or frost. This knowledge can be crucial to assess the potential of selective transplanting of climate-resistant provenances or ecotypes of native or exotic species as a tool of coping and adaptation strategy in agriculture and forestry to dampen the harmful impacts of such extremes in the face of climate change (**Manuscripts 2, 3, 4, 5, 7, 8, 9**). Unlike in economics, ecosystem management has hardly introduced risk management into decision making processes until today, despite the strong risk of an uncertain future in terms of climatic conditions (Knoke et al. 2005; Hanewinkel et al. 2011). In economics, the risk of a complete loss of profits is reduced by a diversification of investments. This effect is called the *portfolio effect* and was described by Markowitz (1952). In ecology, a comparable concept, the insurance hypothesis, describes a the positive effect of biodiversity on ecosystem functioning and reliability, as the higher number of species, the more likely the function of a failing species can be adopted by other species in the system (Yachi and Loreau 1999). The conversion of monocultures into mixed forests, i.e. an increase of species diversity as insurance against adverse biotic and abiotic impactshas become popular over the last decades (Knoke et al. 2005), yet the role that within-species diversity could play in this context just recently came into focus of forest science and management.

With respect to the described uncertainties and the potential positive effects of biodiversity on risk abatement, the mixing of provenances has been suggested by several authors (Kolström et al. 2011; Frascaria-Lacoste and Fernández-Manjarrés 2012). However, evidence has to be provided whether an anthropogenic enhancement of genotypic diversity and phenotypic plasticity, e.g. by intermixing local, highly-adapted and very plastic provenances from

different climatic regions, may maintain high yields under favourable conditions and securing ecosystem functioning, persistence and services under extreme conditions (**Manuscript 8).**

3. Synopsis of the Thesis

3.1. General Objectives and Approach

Considering the challenges that ecosystem management, forestry, agriculture, and nature conservation will have to meet in the face of climate change, the overall objective of this thesis was to

- assess the potential of the selective use of within-species variability (provenances, ecotypes) as a tool for climate change adaptation and to
- identify provenances or ecotypes of key grassland and forest species, which are better adapted to future climate conditions, especially to climatic extreme events, such as prolonged drought periods.

The first Manuscript (**Manuscript 1**) introduces the topic of Assisted Colonization or Assisted Migration of species or ecotypes/provenances and discusses its potential as a tool in climate change adaptation. This is increasingly discussed as a proactive strategy but still there is insufficient knowledge about the prospects of success and risk.

Then the aim was to depict possible future climate conditions for Bayreuth/Germany with the help of regional climate models, such as REMO (BfG 2009) based on the IPCC emission scenario A1B (IPCC 2000). In a second step, regions within the distribution range of the species tested in this study (*Fagus sylvatica*, *Pinus nigra*, *Arrhenatherum elatius*, *Alopecurus pratensis*, *Festuca pratensis*, *and Holcus lanatus*) were identified, where current climate conditions are close to those projected for Bayreuth/Germany for the 2071-2100 period. Seed material was obtained from these regions, where possible, as we assumed a higher adaptedness of these provenances/ecotypes to this specific climatic environment.

In a last step, 1-year old plants of the selected provenances of the above mentioned species were exposed to a temperature treatment (warming and control) and to a precipitation treatment (drought and control). The climate manipulations were fully crossed resulting in four treatments (control, drought, warming, warming & drought), which were replicated three times each, resulting in an experimental design with 12 experimental units (greenhouses). All plants were planted individually in pots.

Figure 2a: Left: Overview of the experimental site with the 12 experimental units (greenhouses).

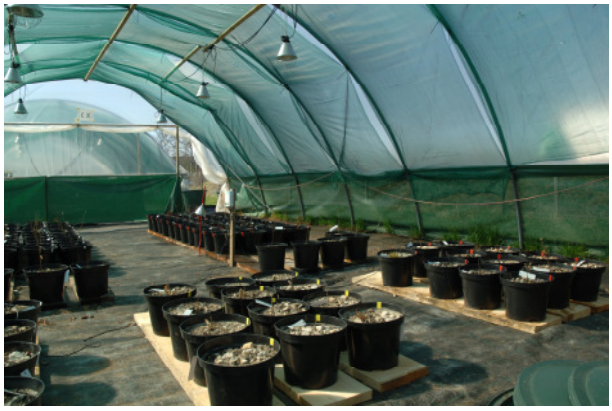

Figure 2b: Inside an experimental unit with warming treatment (wind-shelters and UV-lamps) with potted *F. sylvativa* saplings

Figure 3: Biomass harvest of grassland species in 2009

In 2009 provenances of the four grass species and P. nigra were tested, in order to identify ecotypes that are optimally adapted to the projected future climate conditions (**Manuscripts 2 and 4**).

In year 2010 provenances of *F. sylvatica* were exposed to the same treatments (Nagy et al. **in prep**., not included in this thesis) At the same time a similar experiment was conducted in Landau/Germany with *F. sylvatica*, yet without the warming treatment, but with two different soil types, to asses the interaction of provenance, climate and other abiotic factors (**Manuscript 3**). Landau in Rhineland-Palatinate lies between the Upper Rhine Plain and the Palatinate forest and features an annual mean temperature about 2° above the annual average for Bayreuth. A study in which the Bayreuth experimental warming is compared with the "geographic" warming based on the two test sites is in preparation (not included in this thesis).

Like mentioned in the introduction, late frost events are expected to be still part of a Central European climate even under generally warmer conditions in the future. Therefore, potential candidate provenances or ecotypes that might replace or complement local ones, have to prove that they are able to cope with these frost events. Provenances or ecotypes of the listed grass species and of *Fagus sylvatica* were exposed to a late frost treatment in order to assess whether provenances from less frost-prone sites show a higher late frost sensitivity. Additionally, the impact of preceding warming treatments on late frost sensitivity was tested (**Manuscripts 6, 7**).

Figure 4: Leaf damage after late frost treatment of *F. sylvtica*

Pinus nigra is a non-native coniferous species that is considered to be a potential candidate to replace climate-threatened native coniferous species like *Picea abies*. Yet the main parts of its distribution range are located in the Mediterranean region with less severe winters compared to Central Europe or Germany. Cold hardiness of *P. nigra* provenances was tested in the lab

by the Relative Electrolyte Leakage method (REL) and the influence of the preceding climate treatments on the cold hardiness was determined. Furthermore the frost tolerance of *P. nigra* was compared to the one of native conifers (**Manuscript 5**).

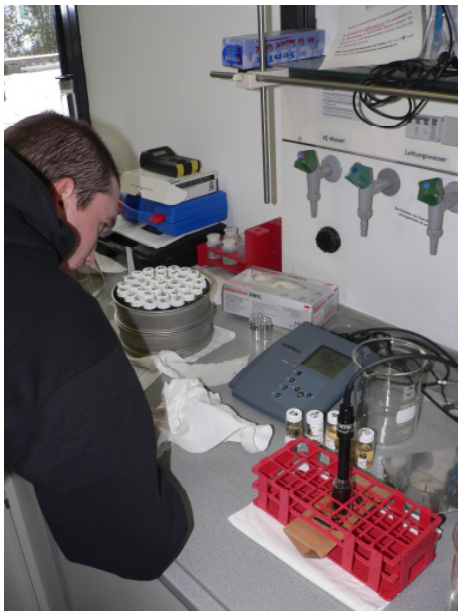

Figure 5: Cold Hardiness measurements (REL) in the lab of provenances of *P. nigra* and other local conifer species.

In the course of this study, no evidence was found that it will be likely to identify one single "best-adapted" and so to say optimal provenance for the future conditions of a specific site such as Bayreuth. Climate change is a moving target, but additionally, provenances exhibit complex response patterns to combined and sequential changes. Rather it seems to be reasonable to enhance genetic diversity and phenotypic plasticity of forest stands, generally. A model was developed in order to test the hypothesis that a mixing of different provenances may reduce the risk of a total breakdown of populations under extreme events or the risk of high yield losses under favourable conditions. The model was fed with increment data of *P. nigra* and *F. sylvatica* from the above mentioned experiments (**Manuscript 8**).

3.2. Remarks on Methodological Challenges

During the study many conceptual and methodological challenges arose. Firstly, when trying to determine the target areas, with climate conditions close to projected ones for Germany and from which to obtain the seed material for the experiments, the problem was to settle on an emission scenario on which projections should be based on. Finally the A1B scenario was used, as this corresponds with the 2.0°C target and is "politically favoured", even if we were

aware of the fact, that actual emissions take course on the trajectories of the most pessimistic scenario. However, these scenarios and the subsequential global models will be modified by the IPCC in the near future.

Furthermore, it is not trivial to determine those climate variables that have to be included in the search of climate-equivalent regions. Mean values are most probably not very meaningful in this context, as they do not reflect e.g. the allocation of precipitation within or outside the growing seasons or the distribution of temperature minima and maxima throughout the year. On the other hand, climate models still have a lot of shortcomings in terms of the projection of climate extremes.

The simulation of extreme events, in this case of the determination of the duration of the drought periods, is another challenge in climate change experiments (Beier et al. 2012). In this study two approaches were used. On was the calculation of duration via extreme value statistics of local climate data based on the reoccurrence probability of a drought event, e.g. a 1000-year drought event. This bears the risk that the event might actually be not extreme for the exposed organisms. The other method was to derive the duration from the reaction of the drought-exposed organisms, e.g. the drought is imposed until a certain percentage of individuals exhibit a certain amount of visible damage. Evidence suggests that the timing, not just the magnitude of extreme events, is extremely important, when assessing their impact on vegetation (Craine et al. 2012). The impact of the timing of a drought period on the response of different provenances of *Quercus pubescens* is currently investigated within this experiment (Backhaus et al. **in prep**., not included in this thesis).

Juvenile plants were used in this experiment (grass individuals were 3 months old at the start of the experiment, tree individuals had an age of 1 year), what bears some problems. One the one hand, the response of juvenile stages of plants to climatic extremes is crucial for a functioning regeneration and recruitment of a stand, especially in forests. On the other hand, the transferability of insights gained from juvenile plants to adult individuals or stands might be limited.

Finally, the decision for the set-up of the experiment as a pot-experiment bears some problems, as well. On the one hand it enables us to impose artificial extreme events on selected individuals, what would be almost impossible, or only feasible with extremely high financial and labour investments in the field. On the other hand it forces us to blind out certain aspects e.g. such as below ground root competition or other interaction between individuals that would occur in a classic provenance trial in forest stands. In general a trade-off exists

between experiments with a close approximation to real conditions and experiments with the ability to strongly control single or combination of factors. The here discussed experiments can be considered to be seen as strongly controlled. Along this trade-off repeated experiments would be necessary to deliver a comprehensive picture of ecological and physiological processes in response to climate change.

All these challenges and problems need further methodological research in order to improve the findings made in future climate experiments, e.g. on the determination and quantification of "extremeness" of an event, on the artefacts of artificial climate manipulations (e.g. Wolkovich et al. (2012) recently claimed that by experimental warming the shift in phenology of plants in response to higer temperatures was underestimated) or on the transferability of climate responses between age classes of plants.

3.3. Main Findings and Conclusion

Differences in the response or sensitivity to climate change and extreme events of different provenances or ecotypes of species were found to be very specific. All four grass species exhibited differnces in their sensitivity to climate manipulation for at least one response parameter (**Manuscript 4**). This implies that certain ecotypes are able to cope better with climate perturbations than others. However, the hypothesis that southern, more drought- or heat-prone ecotypes are better adapted to warm and dry conditions compared to local ecotypes could not be supported. On the basis of this small subset of ecotypes used in the experiment, no evidence of geographic patterns in climate-sensitivity was found.

Also for *F. sylvatica* significant differences in drought tolerance between the investigated provenances were demonstrated (**Manuscript 3**), despite an overall drought-induced growth reduction. Sandy soil conditions amplify the negative impact of drought on growth, compared to more loamy conditions. This indicates the importance of the interaction of climatic variables with other abiotic site-related factors. Furthermore, evidence suggests that a correlation between the drought proneness of the place of origin of the provenances and their performance under drought condition may exist. Additionally, it became evident that drought-adapted provenances, i.e. provenances that show no or only small increment reductions under water limitation, are not necessarily able to compete with less drought-adapted provenances under favourable conditions. Therefore a trade-off between stability and yield can be expected, as highly adapted provenances may secure survival of stands during extreme events, but may not be able to reach high yield rates under better conditions. This may limit the

effectiveness of transplantation of single highly-adapted provenances as a means of climate change adaptation (Richter et al. 2011).

Surprisingly, no differences in response to climate manipulations between the investigated provenances could be demonstrated for *P. nigra* (**Manuscript 2**). Nevertheless, differences in the overall performance between provenances were evident, but no signs of local adaptation to drought and mean temperatures were found. This might be explained by a high drought-tolerance of the species across populations (Lebourgeois et al. 1998; Martinez-Vilalta and Pinol 2002). Interestingly, the impact of the drought period on growth was not significant until the second year, indicating a lag-effect. Comparable responses have already been reported for mature trees (e.g. Lebourgeois 2000; Martin-Benito et al. 2008), but are a new finding for juvenile stages of tree growth. Therefore short-term studies on climate change impacts on plants must take care not to over-interpret initial tolerance to climatic stressors.

Besides the dry and warm extremes frost over winter and spring seems to be crucial for temperate vegetation performance (Kreyling 2010). Local adaptations to these events indicate that frost is an important stressor and impacts species on an evolutionary level.

Grass species suffered during the vegetation period under previous late frost events with up to 20% reduction in aboveground biomass production (**Manuscript 7**). At least one species showed a correlation between May minimum temperatures and reduction in biomass production, indicating an evolutionary adaptation to local conditions. Interactions of late frost sensitivity with preceding warming treatment shows that in a warmer world with an earlier onset of the growing season, late frost damages can even become more frequent and severe (Liu and Osborne 2008). Astonishingly, preceding drought treatment seems to increase late frost tolerance for one species, providing evidence for the relatedness of physiological mechanisms that are responsible for drought and frost tolerance (Beck et al. 2007).

The late-frost experiment with *Fagus sylvatica* yielded comparable results for this important key species (**Manuscript 6**). Late-frost increased leaf injury and reduced increment, with significant differences in late frost sensitivity between the provenances on a regional and continental scale, and local adaptations to May minimum temperatures. As stated above, late frost damages are expected to increase in a warmer world as frost events may hit the vegetation during a growing period that will start earlier (Kramer et al. 2000). However, results from this experiment point to the importance of the timing of such events. Here, the warming treatment led to an earlier maturation of leaves. The late frost event impacted these

plants less, as the leaves seemed to be hardened out and become less sensitive than the non-warming plants whose buds just burst before the frost event.

Pinus nigra is discussed as a potential substitute for native conifers at dry sites in Central Europe. Therefore it is important to know whether this species, with a distribution range in warmer regions of the (sub-)Mediterranean area will survive harsher winters in Central Europe and whether intra-specific difference between provenances exist (**Manuscript 5**). Our results show that *P. nigra* provenances strongly vary in terms of winter frost hardiness and that local adaptation to winter conditions can be detected. Both severe drought events and summer warming can increase cold hardiness, indicating that the interaction between different climatic events can lead to unexpected responses and that winter survival is influenced by climatic events during the growing season. In general, *P. nigra* (or at least the selected provenances of this species) seems to be well adapted for cultivation in temperate regions.

Given the multitude of climatic factors, and potential trade-offs between adaptedness and plasticity, the recently proposed (e.g. mixing of provenances as insurance against an unsecure climatic future, was tested by a modelling process based on increment data of *P. nigra* and *F. sylvatica* (**Manuscript 8**). Our results imply that enhancing intraspecific diversity by mixing of provenances cannot impede the risk of yield losses, but increase the chance of high yields. Therefore promoting the diversity of provenances might be one strategy to cope with adverse impacts of climate change, considering the associated uncertainties.

In general, the findings of this study show that the selective use of plant provenances and of within-species variability has a potential to contribute to the mitigation of adverse impacts of climate change and extreme events on ecologically and economically important ecosystems, such as forest and permanent grasslands. However, taking into account the uncertainties of future emission scenarios and those inherent in climate models, and the multitude of climatic and abiotic factors affecting performance of a species, however, it seems not to be very realistic to look for one single “best-adapted” provenance or ecotype. Furthermore there might be a trade-off between adaptation to stress factors and phenotypic plasticity, what means that concentrating on a single highly-adapted provenance or ecotype might lead to decline in performance under favourable conditions and therefore limit the effectiveness of such measures (Richter et al. 2012).

First of all the difficulty of choosing an adequate emission scenario and the model-inherent uncertainties of climate predictions, especially in terms of precipitation extremes, limits the

prospects of success of such a strategy. Moreover, provenances or ecotypes that are well-adapted to certain extreme climatic events (e.g. drought) may be sensitive to other climatic factors, such as late frosts.

The timing and interaction of several climate factors in a changing climate, especially on the background of warmer average conditions and longer growing seasons, proofed to be of importance, as they can magnify or reduce the impact of each single factor, and need more attention in future climate change experiments. Likewise non-climatic factors, such as soil properties or biotic factors, such as herbivores or inter-specific competition interact with a changing climatic environment. Especially the integration of biotic antagonists and other distrurbances will be one of the major challenges of future climate change experiments (Beier et al. 2012).

It could be demonstrated that some of the effects of climate extremes on the performance of plant species can be substantially delayed. Ecological inertia must be considered. Responses can become evident one year after the actual extreme event took place, as the events may alter e.g. soil processes that can have delayed and long-term impacts on ecosystems, what underlines the importance of the establishment of long-term experiments and observations.

The mixing of provenances and thus the facilitation of genetic diversity and phenotypic plasticity is one promising attempt for climate adaptation in ecosystem management. Our results suggest that the more provenances are included, the higher the chances of having a "best-performer" in your portfolio of ecotypes for each possible climate event. Furthermore genetic diversity and phenotypic plasticity foster the capacity of rapid evolutionary adaptation to changing environments. However, this strategy comes not without any risks, as the introduction of new genetic material may lead to outbreeding depression or the loss of genetic diversity across the entire distribution range of species in future plant generations.

Nonetheless, the maintenance and support of intra-specific diversity is important in the face of changing environmental conditions, and should consequently be included in future climate change and adaptation research, as the adaptation capability of species might else be misinterpreted.

4. References of Introduction and Synopsis

Aguinagalde, I.; Llorente, F.; Benito, C. (1997): Relationships among five populations of European black pine (*Pinus nigra ARN.*) using morphometric and isozyme markers. In: *SILVAE GENETICA* 46. 1–5.

Alexander, L. V.; Zhang, X.; Peterson, T. C.; Caesar, J.; Gleason, B.; Tank, AMGK et al. (2006): Global observed changes in daily climate extremes of temperature and precipitation. In: *JOURNAL OF GEOPHYSICAL RESEARCH-ATMOSPHERES* 111 (D5).

Alkemade, R.; Bakkenes, M.; Eickhout, B. (2011): Towards a general relationship between climate change and biodiversity: an example for plant species in Europe. In: *REGIONAL ENVIRONMENTAL CHANGE* 11.143-150.

Allen, C.D.; Macalady, A.K.; Chenchouni, H.; Bachelet, D.; McDowell, N.; Vennetier, M. et al. (2010): A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. In: *FOREST ECOLOGY AND MANAGEMENT* 259. 660–684.

Andreu, L.; Gutierrez, E.; Macias, M.; Ribas, M.; Bosch, O.; Camarero, J. (2007): Climate increases regional tree-growth variability in Iberian pine forests. In: *GLOBAL CHANGE BIOLOGY* 13. 804-815.

Bakkenes, M.; Alkemade, J. R.M.; Ihle, F.; Leemans, R.; Latour, J. B. (2002): Assessing effects of forecasted climate change on the diversity and distribution of European higher plants for 2050. In: *GLOBAL CHANGE BIOLOGY* 8. 390–407.

Ballester, J.; Rodo, X.; Giorgi, F. (2010): Future changes in Central Europe heat waves expected to mostly follow summer mean warming. In: *CLIMATE DYNAMICS* 35. 1191–1205.

Bartolini, G.; Di Stefano, V.; Maracchi, G.; Orlandini, S. (2012): Mediterranean warming is especially due to summer season Evidences from Tuscany (central Italy). In: *THEORETICAL AND APPLIED CLIMATOLOGY* 107. 279–295.

Beck, E.H.; Fettig, S.; Knake, C.; Hartig, K.; Bhattarai, T. (2007): Specific and unspecific responses of plants to cold and drought stress. In: *JOURNAL OF BIOSCIENCES* 32 (3), S. 501–510.

Beier, C.; Beierkuhnlein, C.; Wohlgemuth, T.; Penuelas, J.; Emmett, B. (2012): Precipitation manipulation experiments – challenges andrecommendations for the future. In: *ECOLOGY LETTERS* doi: 10.1111/j.1461-0248.2012.01793.x

Beierkuhnlein, C.; Foken T. (2008): Klimawandel in Bayern. Auswirkungen und Anpassungsmöglichkeiten. Bayreuther Forum Ökologie, 113. 2-44.

Beniston, M.; Stephenson, D.; Christensen, O.; Ferro, C.; Frei, C.; Goyette, S. et al. (2007): Future extreme events in European climate: an exploration of regional climate model projections. In: *CLIMATIC CHANGE* 81. 71–95.

Betsch, P.; Bonal, D.; Breda, N.; Montpied, P.; Peiffer, M.; Tuzet, A.; Granier, A. (2011): Drought effects on water relations in beech: The contribution of exchangeable water reservoirs. In: *AGRICULTURAL AND FOREST METEOROLOGY* 151. 531–543.

BfG (2009): *REMO-BFG Version 5.8.* Max Planck Institut for Meteorology on behalf of the Bundesanstalt für Gewässerkunde (BfG), Am Mainzer Tor 1, D-56068, Koblenz, Germany.

Biel, C.; Save, R.; Habrouk, A.; Espelta, J. M.; Retana, J. (2004): Effects of restricted watering and CO2 enrichment in the morphology and performance after transplanting of nursery-grown *Pinus nigra* seedlings. In: *HORTSCIENCE* 39. 535–540.

Blenkinsop, S.; Fowler, H. J. (2007): Changes in European drought characteristics projected by the PRUDENCE regional climate models. In: *INTERNATIONAL JOURNAL OF CLIMATOLOGY* 27. 1595–1610.

Boeck, H. J. de; Lemmens, C. M. H. M.; Gielen, B.; Bossuyt, H.; Malchair, S.; Carnol, M. et al. (2007): Combined effects of climate warming and plant diversity loss on above- and below-ground grassland productivity. In: *ENVIRONMENTAL AND EXPERIMENTAL BOTANY* 60. 95–104.

Boeck, H.J. de; Dreesen, F.E.; Janssens, I.A.; Nijs, I. (2011): Whole-system responses of experimental plant communities to climate extremes imposed in different seasons. In: *NEW PHYTOLOGIST* 189. 806–817.

Bolte, A.; Ammer, C.; Lof, M.; Madsen, P.; Nabuurs, G.; Schall, P. et al. (2009): Adaptive forest management in central Europe: Climate change impacts, strategies and integrative concept. In: *SCANDINAVIAN JOURNAL OF FOREST RESEARCH* 24. 473–482.

Bolte, A.; Hilbrig, L.; Grundmann, B.; Kampf, F.; Brunet, J.; Roloff, A. (2010): Climate change impacts on stand structure and competitive interactions in a southern Swedish spruce-beech forest. In: *EUROPEAN JOURNAL OF FOREST RESEARCH* 129. 261–276.

Bolte, A.; Degen, B. (2010): Forest adaptation to climate change - options and limitations. In: *LANDBAUFORSCHUNG* 60. 111–117.

Bonn, S. (2000): Competition dynamics in mixed beech-oak stands and its modifications expected due to climate changes. In: *ALLGEMEINE FORST UND JAGDZEITUNG* 171. 81–88.

Briffa, K. R.; van der Schrier, G.; Jones, P.D. (2009): Wet and dry summers in Europe since 1750: evidence of increasing drought. In: *INTERNATIONAL JOURNAL OF CLIMATOLOGY* 29. 1894–1905.

Brohan, P.; Kennedy, J.J.; Harris, I.; Tett, S. F.B.; Jones, P.D. (2006): Uncertainty estimates in regional and global observed temperature changes: A new data set from 1850. In: *JOURNAL OF GEOPHYSICAL RESEARCH-ATMOSPHERES* 111 (D12).

Cattiaux, J.; Vautard, R.t; Yiou, P. (2011): North-Atlantic SST amplified recent wintertime European land temperature extremes and trends. In: *CLIMATE DYNAMICS* 36. 2113–2128.

Chen, I.; Hill, J.K.; Ohlemueller, R.; Roy, D.B.; Thomas, C.D. (2011): Rapid Range Shifts of Species Associated with High Levels of Climate Warming. In: *SCIENCE* 333. 1024–1026.

Chen, P.Y.; Welsh, C.; Hamann, A. (2010): Geographic variation in growth response of Douglas-fir to interannual climate variability and projected climate change. In: *GLOBAL CHANGE BIOLOGY* 16. 3374–3385.

Ciais, P.; Reichstein, M.; Viovy, N.; Granier, A.; Ogee, J.; Allard, V. et al. (2005): Europe-wide reduction in primary productivity caused by the heat and drought in 2003. In: *NATURE* 437. 529–533.

Clark, J.S.; Bell, D.M.; Hersh, M.H.; Nichols, L. (2011): Climate change vulnerability of forest biodiversity: climate and competition tracking of demographic rates. In: *GLOBAL CHANGE BIOLOGY* 17. 1834–1849.

Comps, B.; Thiebaut, B.; Paule, L.; Merzeau, D.; Letouzey, J. (1990): Allozymic variability in beechwoods (*Fagus sylvatica L.*) over Central Europe – Spatial differentiation among and within populations. In: *HEREDITY* 65. 407–417.

Craine, J.M.; Nippert, J.B.; Elmore, A.J.; Skibbe, A.M.; Hutchinson, S.L.; Brunsell, N. A. (2012): Timing of climate variability and grassland productivity. In: *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* 109. 3401–3405.

Czajkowski, T.; Kuhling, M.; Bolte, A. (2005): Impact of the 2003 summer drought on growth of beech sapling natural regeneration (*Fagus sylvatica L.*) in north-eastern Central Europe. In: *ALLGEMEINE FORST UND JAGDZEITUNG* 176. 133–143.

Czajkowski, T.; Bolte, A. (2006): Different reaction of beech (*Fagus sylvatica L.*) provenances from Germany and Poland to drought. In: *ALLGEMEINE FORST UND JAGDZEITUNG* 177. 30–40.

Czucz, B.; Galhidy, L.; Matyas, C. (2011): Present and forecasted xeric climatic limits of beech and sessile oak distribution at low altitudes in Central Europe. In: *ANNALS OF FOREST SCIENCE* 68. 99–108.

Dai, A. (2011): Characteristics and trends in various forms of the Palmer Drought Severity Index during 1900-2008. In: *JOURNAL OF GEOPHYSICAL RESEARCH-ATMOSPHERES* 116.

Della-Marta, P.M.; Haylock, M.R.; Luterbacher, J.; Wanner, H. (2007): Doubled length of western European summer heat waves since 1880. In: *JOURNAL OF GEOPHYSICAL RESEARCH-ATMOSPHERES* 112 (D15).

Desprez-Loustau, M.; Robin, C.; Reynaud, G.; Deque, M.; Badeau, V.; Piou, D. et al. (2007): Simulating the effects of a climate-change scenario on the geographical range and activity of forest-pathogenic fungi. In: *CANADIAN JOURNAL OF PLANT PATHOLOGY-REVUE CANADIENNE DE PHYTOPATHOLOGIE* 29. 101–120.

Easterling D.R.; Meehl, G.A.; Parmesan, C.; Changnon, S A.; Karl, T.R.; Lo Mearns (2000): Climate extremes: Observations, modeling, and impacts. In: *SCIENCE* 289. 2068–2074.

EUROSTAT (2010) http://epp.eurostat.ee.europa.eu/statistics_explained/index.php/Main_Page.

Fetcher, N.; Shaver, G.R. (1990): Environmental sensitivity of ecotypes as a potential influence on primary production. In: *AMERICAN NATURALIST* 136. 126–131.

Fischer, E.M.; Schaer, C. (2009): Future changes in daily summer temperature variability: driving processes and role for temperature extremes. In: *CLIMATE DYNAMICS* 33. 917–935.

Fotelli, M.N.; Gessler, A.; Peuke, A.D.; Rennenberg, H. (2001): Drought affects the competitive interactions between *Fagus sylvatica* seedlings and an early successional species, *Rubus fruticosus*: responses of growth, water status and delta C-13 composition. In: *NEW PHYTOLOGIST* 151. 427–435.

Fotelli, M.N.; Nahm, M.; Radoglou, K.; Rennenberg, H.; Halyvopoulos, G.; Matzarakis, A. (2009): Seasonal and interannual ecophysiological responses of beech (*Fagus sylvatica*) at its south-eastern distribution limit in Europe. In: *FOREST ECOLOGY AND MANAGEMENT* 257. 1157–1164.

Frascaria-Lacoste, N.; Fernández-Manjarrés, J. (2012): Assisted Colonization of Foundation Species: Lack of Consideration of the Extended Phenotype Concept—Response to Kreyling et al. (2011). In: *RESTORATION ECOLOGY* 20. 296-298.

Friedrichs, D.A.; Trouet, V.; Buentgen, U.; Frank, D.C.; Esper, J.; Neuwirth, B.; Loeffler, J. (2009): Species-specific climate sensitivity of tree growth in Central-West Germany. In: *TREES-STRUCTURE AND FUNCTION* 23. 729–739.

Gessler, A.; Keitel, C.; Kreuzwieser, J.; Matyssek, R.; Seiler, W.; Rennenberg, H. (2007): Potential risks for European beech (*Fagus sylvatica L.*) in a changing climate. In: *TREES-STRUCTURE AND FUNCTION* 21. 1–11.

Gielen, B.; Naudts, K.; D'Haese, D.; Lemmens, C.; Boeck, Hans J. de; Biebaut, Eddy et al. (2007): Effects of climate warming and species richness on photochemistry of grasslands. In: *PHYSIOLOGIA PLANTARUM* 131. 251–262.

Gu, L.; Hanson, P.J.; Mac Post, W.; Kaiser, D.P.; Yang, B.; Nemani, R. et al. (2008): The 2007 eastern US spring freezes: Increased cold damage in a warming world? In: *BIOSCIENCE* 58. 253–262.

Gutschick, V.P.; BassiriRad, H. (2003): Extreme events as shaping physiology, ecology, and evolution of plants: toward a unified definition and evaluation of their consequences. In: *NEW PHYTOLOGIST* 160. 21–42.

Hanewinkel, M.; Hummel, S.; Albrecht, A. (2011): Assessing natural hazards in forestry for risk management: a review. In: *EUROPEAN JOURNAL OF FOREST RESEARCH* 130. 329–351.

Hanninen, H. (2006): Climate warming and the risk of frost damage to boreal forest trees: identification of critical ecophysiological traits. In: *TREE PHYSIOLOGY* 26. 889–898.

Hegerl, G.C.; Hanlon, H.; Beierkuhnlein, C. (2011): Climate Science: Elusive Extremes. In: NATURE GEOSCIENCE 4. 142-142.

Hemery, G. E. (2008): Forest Management and Silvicultural Responses to Projected Climate Change Impacts on European Broadleaved Trees and Forests. . In: *INTERNATIONAL FORESTRY REVIEW* 10. 591–607.

Hewitt, N.; Klenk, N.; Smith, A.L.; Bazely, D.R.; Yan, N.; Wood, S. et al. (2011): Taking stock of the assisted migration debate. In: *BIOLOGICAL CONSERVATION* 144. 2560–2572.

Hlasny, T.; Barcza, Z.; Fabrika, M.; Balazs, B.; Churkina, G.; Pajtik, J. et al. (2011): Climate change impacts on growth and carbon balance of forests in Central Europe. In: *CLIMATE RESEARCH* 47. 219–236.

Huber G. (2011): Neue Tests für Schwarzkiefern-Herkünfte in Bayern im Hinblick auf den Klimawandel. Internationaler Bayerischer Schwarzkiefern-Herkunftsversuch 2009/2010. Teisendorf.

Hufford, K.M.; Mazer, S J. (2003): Plant ecotypes: genetic differentiation in the age of ecological restoration. In: *TRENDS IN ECOLOGY & EVOLUTION* 18. 147–155.

Huntington, T.G. (2006): Evidence for intensification of the global water cycle: Review and synthesis. In: *JOURNAL OF HYDROLOGY* 319. 83–95.

Inouye, D.W. (2008): Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. In: *ECOLOGY* 89. 353–362.

IPCC (2000): Special report on emission scenarios.

IPCC (2012): Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change [Field, C.B., V. Barros, T.F. Stocker, D. Qin, D.J. Dokken, K.L. Ebi, M.D. Mastrandrea, K.J. Mach, G.-K. Plattner, S.K. Allen, M. Tignor, and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, UK, and New York, NY, USA, 582 pp.

Isajev V., Fady B. Semerci H. Andonovski V. (2004): EUFORGEN Technical guidelines for the genetic conservation and use of European Black pine (Pinus nigra). International Plant Genetic resources Institute. Rome, Italy.

Jagielska, A.; Cwalina, M.; Prus-Glowacki, W. (2007): Genetic diversity of the black pine's stands (*Pinus nigra Arn.*). In: *SYLWAN* 151. 23–31.

Jentsch, A.; Kreyling, J.; Elmer, M.; Gellesch, E.; Glaser, B.; Grant, K. et al. (2011): Climate extremes initiate ecosystem-regulating functions while maintaining productivity. In: *JOURNAL OF ECOLOGY* 99. 689–702.

Jentsch, A.; Beierkuhnlein, C. (2008): Research frontiers in climate change: Effects of extreme meteorological events on ecosystems. In: *COMPTES RENDUS GEOSCIENCE* 340. 621–628.

Jentsch, A.; Beierkuhnlein, C. (2010): Simulating the future - responses of ecosystems, key species and European provenances to expected climatic trends and events, *NOVA ACTA LEOPOLDINA* 112. 89-98.

Jentsch, A.; Kreyling, J.; Boettcher-Treschkow, J.; Beierkuhnlein, C. (2009): Beyond gradual warming: extreme weather events alter flower phenology of European grassland and heath species. In: *GLOBAL CHANGE BIOLOGY* 15. 837–849.

Jentsch, A.; Kreyling, J.; Beierkuhnlein, C.(2007): A new generation of climate-change experiments: events, not trends. In: *FRONTIERS IN ECOLOGY AND THE ENVIRONMENT* 5. 365–374.

Joshi, J.; Schmid, B.; Caldeira, M.C.; Dimitrakopoulos, P G.; Good, J.; Harris, R. et al. (2001): Local adaptation enhances performance of common plant species. In: *ECOLOGY LETTERS* 4. 536–544.

Jump, A.S.; Hunt, J.M.; Penuelas, J. (2006): Rapid climate change-related growth decline at the southern range edge of Fagus sylvatica. In: *GLOBAL CHANGE BIOLOGY* 12. 2163–2174.

Jung, T. (2009): Beech decline in Central Europe driven by the interaction between *Phytophthora* infections and climatic extremes. In: *FOREST PATHOLOGY* 39. 73–94.

Jylhä, K.; Fronzek, S.; Tuomenvirta, H.; Carter, T.; Ruosteenoja, K. (2008): Changes in frost, snow and Baltic Sea ice by the end of the twenty-first century based on climate model projections for Europe. In: *CLIMATIC CHANGE* 86. 441–462.

Kardol, P.; Campany, C.E.; Souza, L.; Norby, R.J.; Weltzin, J.F.; Classen, A.T. (2010): Climate change effects on plant biomass alter dominance patterns and community evenness in an experimental old-field ecosystem. In: *GLOBAL CHANGE BIOLOGY* 16. 2676–2687.

Kioutsioukis, I.; Melas, D.; Zerefos, C. (2010): Statistical assessment of changes in climate extremes over Greece (1955-2002). In: *INTERNATIONAL JOURNAL OF CLIMATOLOGY* 30. 1723–1737.

Kjellstrom, E.; Barring, L.; Jacob, D.; Jones, R.; Lenderink, G.; Schaer, C. (2007): Modelling daily temperature extremes: recent climate and future changes over Europe. In: *CLIMATIC CHANGE* 81. 249–265.

Knoke, T.; Stimm, B.; Ammer, C.; Moog, M. (2005): Mixed forests reconsidered: A forest economics contribution on an ecological concept. In: *FOREST ECOLOGY AND MANAGEMENT* 213. 102–116.

Kodra, E.; Steinhaeuser, K.; Ganguly, A.R. (2011): Persisting cold extremes under 21st-century warming scenarios. In: *GEOPHYSICAL RESEARCH LETTERS* 38.

Kölling C. (2007): Klimahüllen für 27 Waldbaumarten. In: *AFZ DER WALD* 23. 1242–1245.

Kölling C.; Knoke T.; Schall P.; Ammer C. (2009): Überlegungen zum Risiko des Fichtenanbaus in Deutschland vor dem Hintergrund des Klimawandels. In: *FORSTARCHIV* 80. 42–54.

Kolström, M.; Vilen, T.; Lindner, M. (2011) Climate Change Impacts and Adaptation in European Forstes. In: Tikkanen, I.; Päivinen, R.; Korhonen, M. (Ed.): EFI Policy Brief 6, European Forest Institute, Joensuu.

Konnert, M. (1995): Investigations on the genetic variation of beech (*Fagus sylvatica L*) in Bavaria. In: *SILVAE GENETICA* 44. 346–351.

Kramer, K.; Leinonen, I.; Loustau, D. (2000): The importance of phenology for the evaluation of impact of climate change on growth of boreal, temperate and Mediterranean forests ecosystems: an overview. In: *INTERNATIONAL JOURNAL OF BIOMETEOROLOGY* 44. 67–75.

Kreyling, J. (2010): Winter climate change: a critical factor for temperate vegetation performance. In: *ECOLOGY* 91. 1939–1948.

Kreyling, J.; Beierkuhnlein, C.; Elmer, M.; Pritsch, K.; Radovski, M.; Schloter, M. et al. (2008a): Soil biotic processes remain remarkably stable after 100-year extreme weather events in experimental grassland and heath. In: *PLANT AND SOIL* 308. 175–188.

Kreyling, J.; Stahlmann, R.; Beierkuhnlein, C. (2012): Räumliche Variation in der Blattschädigung von Waldbäumen nach dem extremen Spätfrostereignis im Mai 2011 (Spatial variation in leaf damage of forest trees after the extreme spring frost event in May 2011). In: *ALLGEMEINE FORST UND JAGDZEITUNG*, 183. 15-22

Kreyling, J.; Wenigmann, M.; Beierkuhnlein, C.; Jentsch, A. (2008b): Effects of extreme weather events on plant productivity and tissue die-back are modified by community composition. In: *ECOSYSTEMS* 11. 752–763.

Kuerbis, K.; Mudelsee, M.; Tetzlaff, G.; Brazdil, R. (2009): Trends in extremes of temperature, dew point, and precipitation from long instrumental series from central Europe. In: *THEORETICAL AND APPLIED CLIMATOLOGY* 98. 187–195.

Kysely, J.; Gaal, L.; Beranova, R.; Plavcova, E. (2011): Climate change scenarios of precipitation extremes in Central Europe from ENSEMBLES regional climate models. In: *THEORETICAL AND APPLIED CLIMATOLOGY* 104. 529–542.

Le Quere, C.; Raupach, M.R.; Canadell, J.G.; Marland et al. (2009): Trends in the sources and sinks of carbon dioxide. In: *NATURE GEOSCIENCE* 2. 831–836.

Lebourgeois, F. (2000): Climatic signals in earlywood, latewood and total ring width of Corsican pine from western France. In: *ANNALS OF FOREST SCIENCE* 57. 155–164.

Lebourgeois, F.; Breda, N.; Ulrich, E.; Granier, A. (2005): Climate-tree-growth relationships of European beech (*Fagus sylvatica L.*) in the French Permanent Plot Network (RENECOFOR). In: *TREES-STRUCTURE AND FUNCTION* 19. 385–401.

Lebourgeois, F.; Levy, G.; Aussenac, G.; Clerc, B.; Willm, F. (1998): Influence of soil drying on leaf water potential, photosynthesis, stomatal conductance and growth in two black pine varieties. In: *ANNALES DES SCIENCES FORESTIERES* 55. 287–299.

Leemans, R.; Eickhout, B.; Strengers, B.; Bouwman, L.; Schaeffer, M. (2002): The consequences of uncertainties in land use, climate and vegetation responses on the terrestrial carbon. In: *SCIENCE IN CHINA SERIES C-LIFE SCIENCES* 45. 126ff.

Lenton, T.M.; Held, H.; Kriegler, E.; Hall, J.W.; Lucht, W.; Rahmstorf, S.; Schellnhuber, H.J. (2008): Tipping elements in the Earth‘s climate system. In: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 105. 1786–1793.

Leuzinger, S.; Zotz, G.; Asshoff, R.; Korner, C. (2005): Responses of deciduous forest trees to severe drought in Central Europe. In: *TREE PHYSIOLOGY* 25. 641–650.

Lindner, M.; Maroschek, M.; Netherer, S.; Kremer, A.; Barbati, A.; Garcia-Gonzalo, J. et al. (2010): Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. In: *FOREST ECOLOGY AND MANAGEMENT* 259. 698–709.

Liu, Mei-Zhen; Osborne, Colin P. (2008): Leaf cold acclimation and freezing injury in C(3) and C(4) grasses of the Mongolian Plateau. In: *JOURNAL OF EXPERIMENTAL BOTANY* 59. 4161–4170.

López-Moreno, J.I.; Vicente-Serrano, S.M.; Angulo-Martínez, M.; Beguería, S.; Kenawy, A. (2010): Trends in daily precipitation on the northeastern Iberian Peninsula, 1955–2006. In: *INTERNATIONAL JOURNAL OF CLIMATOLOGY* 30. 1026-1041.

Macel, M.; Lawson, C.S.; Mortimer, S.R.; Smilauerova, M.; Bischoff, A.; Cremieux, L. et al. (2007): Climate vs. soil factors in local adaptation of two common plant species. In: *ECOLOGY* 88. 424–433.

Magri, D.; Vendramin, G. G.; Comps, B.; Dupanloup, I.; Geburek, T.; Gomory, D. et al. (2006): A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. In: *NEW PHYTOLOGIST* 171. 199–221.

Mann, M.E.; Zhang, Z.; Hughes, M.K.; Bradley, R.S.; Miller, S.K.; Rutherford, S.; Ni, F. (2008): Proxy-based reconstructions of hemispheric and global surface temperature variations over the past two millennia. In: *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* 105. 13252-13257.

Maracchi, G.; Sirotenko, O.; Bindi, M. (2005): Impacts of present and future climate variability on agriculture and forestry in the temperate regions: Europe. In: *CLIMATIC CHANGE* 70. 117–135.

Markowitz, H. (1952): Portfolio selection. In*: JOURNAL OF FINANCE* 7. 77-91.

Martin-Benito, D.; Cherubini, P.; del Rio, M.; Canellas, I. (2008): Growth response to climate and drought in *Pinus nigra Arn*. trees of different crown classes. In: *TREES-STRUCTURE AND FUNCTION* 22. 363–373.

Martin-Benito, D.; del Rio, M.; Canellas, I. (2010): Black pine (*Pinus nigra Arn.*) growth divergence along a latitudinal gradient in Western Mediterranean Mountains. In: *ANNALS OF FOREST SCIENCE* 67.

Martinez-Vilalta, J.; Pinol, J. (2002): Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. In: *FOREST ECOLOGY AND MANAGEMENT* 161. 247–256.

Matyas, C.; Bozic, G.; Goemoery, D.; Ivankovic, M.; Rasztovits, E. (2009): Juvenile growth response of European beech (*Fagus sylvatica L.*) to sudden change of climatic environment in SE European trials. In: *IFOREST-BIOGEOSCIENCES AND FORESTRY* 2. 213–220.

Maxime, C.; Hendrik, D. (2011): Effects of climate on diameter growth of co-occurring *Fagus sylvatica* and Abies alba along an altitudinal gradient. In: *TREES-STRUCTURE AND FUNCTION* 25. 265–276.

Meehl, G. A.; Karl, T.; Easterling; Changnon, S.; Pielke, R.; Changnon, D. et al. (2000): An introduction to trends in extreme weather and climate events: Observations, socioeconomic impacts, terrestrial ecological impacts, and model projections. In: *BULLETIN OF THE AMERICAN METEOROLOGICAL SOCIETY* 81. 413–416.

Menzel, A.; Sparks, T.; Estrella, N; Koch, E.; Aasa, A.; Ahas, R. et al. (2006): European phenological response to climate change matches the warming pattern. In: *GLOBAL CHANGE BIOLOGY* 12. 1969–1976

Michalski, S.G.; Durka, W.; Jentsch, A.; Kreyling, J.; Pompe, S.; Schweiger, O. et al. (2010): Evidence for genetic differentiation and divergent selection in an autotetraploid forage grass (*Arrhenatherum elatius*). In: *THEORETICAL AND APPLIED GENETICS* 120. 1151–1162.

Min, S.-K.; Zhang, X.; Zwiers, F.W.; Hegerl, G.C. (2011): Human contribution to more-intense precipitation extremes. In: *NATURE* 470. 378–381.

Moberg, A.; Jones, P.D.; Lister, D.; Walther, A.; Brunet, M.; Jacobeit, J. et al. (2006): Indices for daily temperature and precipitation extremes in Europe analyzed for the period 1901-2000. In: *JOURNAL OF GEOPHYSICAL RESEARCH-ATMOSPHERES* 111 (D22).

Morecroft, M.D.; Masters, G.J.; Brown, V.K.; Clarke, I.P.; Taylor, M.E.; Whitehouse, A.T. (2004): Changing precipitation patterns alter plant community dynamics and succession in ex-arable grassland. In: *FUNCTIONAL ECOLOGY* 18. 648–655.

Morgan, J. A.; de Pataki; Korner, C.; Clark, H.; Del Grosso, S. J.; Grunzweig, J. M. et al. (2004): Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO2. In: *OECOLOGIA* 140. 11–25.

Murphy, Helen T.; VanDerWal, Jeremy; Lovett-Doust, Jon (2010): Signatures of range expansion and erosion in eastern North American trees. In: *ECOLOGY LETTERS* 13. 1233–1244.

Naudts, K.; van den Berge, J.; Janssens, I. A.; Nijs, I.; Ceulemans, R. (2011): Does an extreme drought event alter the response of grassland communities to a changing climate? In: *ENVIRONMENTAL AND EXPERIMENTAL BOTANY* 70. 151–157.

Nemani, R.R.; Keeling, C.D.; Hashimoto, H.; Jolly, W.M.; Piper, S.C.; Tucker, C.J. et al. (2003): Climate-driven increases in global terrestrial net primary production from 1982 to 1999. In: *SCIENCE* 300. 1560–1563.

Ofir, M; Kigel, J. (2010): Ecotypic variation of summer dormancy relaxation associated with rainfall gradient in the geophytic grass Poa bulbosa. In: *ANNALS OF BOTANY* 105. 617–625.

Ohlemuller, R.; Gritti, E.S.; Sykes, M.T.; Thomas, C.D. (2006): Quantifying components of risk for European woody species under climate change. In: *GLOBAL CHANGE BIOLOGY* 12. 1788–1799.

Orlowsky, B.; Seneviratne, S. (2012): Global changes in extreme events: regional and seasonal dimension. In: *CLIMATIC CHANGE* 110. 669–696.

Parmesan, C.; Yohe, G. (2003): A globally coherent fingerprint of climate change impacts across natural systems. In: *NATURE* 421. 37–42.

Penuelas, J.; Gordon, C.; Llorens, L.; Nielsen, T.; Tietema, A.; Beier, C. et al. (2004): Nonintrusive field experiments show different plant responses to warming and drought among sites, seasons, and species in a north-south European gradient. In: *ECOSYSTEMS* 7. 598–612.

Peuke, A.D.; Gessler, A.; Rennenberg, H. (2006): The effect of drought on C and N stable isotopes in different fractions of leaves, stems and roots of sensitive and tolerant beech ecotypes. In: *PLANT CELL AND ENVIRONMENT* 29. 823–835.

Piovesan, G.; Biondi, F.; Di Filippo, A.; Alessandrini, A.; Maugeri, M. (2008): Drought-driven growth reduction in old beech (*Fagus sylvatica L.*) forests of the central Apennines, Italy. In: *GLOBAL CHANGE BIOLOGY* 14. 1265–1281.

Rennenberg, H.; Seiler, W.; Matyssek, R.; Gessler, A.; Kreuzwieser, J. (2004): European beech (*Fagus sylvatica L.*) - a forest tree without future in the south of Central Europe? In: *ALLGEMEINE FORST UND JAGDZEITUNG* 175. 210–224.

Richter, S.; Kipfer, T.; Wohlgemuth, T.; Calderón Guerrero, C.; Ghazoul, J.; Moser, B.(2012): Phenotypic plasticity facilitates resistance to climate change in a highly variable environment. In: *OECOLOGIA* http://dx.doi.org/10.1007/s00442-011-2191-x.

Rigby, J.R.; Porporato, A. (2008): Spring frost risk in a changing climate. In: *GEOPHYSICAL RESEARCH LETTERS* 35 (12).

Robson, T.M.; Rodriguez-Calcerrada, J.; Sanchez-Gomez, D.; Aranda, I. (2009): Summer drought impedes beech seedling performance more in a sub-Mediterranean forest understory than in small gaps. In: *TREE PHYSIOLOGY* 29. 249–259.

Rose, L.; Leuschner, C.; Koeckemann, B.; Buschmann, H. (2009): Are marginal beech (*Fagus sylvatica L.*) provenances a source for drought tolerant ecotypes? In: *EUROPEAN JOURNAL OF FOREST RESEARCH* 128. 335–343.

Rowell, D.P. (2009): Projected Midlatitude Continental Summer Drying: North America versus Europe. In: *JOURNAL OF CLIMATE* 22. 2813–2833.

Rustad, L.E.; Campbell, J.L.; Marion, G.M.; Norby, R. .; Mitchell, M.J.; Hartley, A.E. et al. (2001): A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. In: *OECOLOGIA* 126. 543–562.

Ryser, P.; Aeschlimann, U. (1999): Proportional dry-mass content as an underlying trait for the variation in relative growth rate among 22 Eurasian populations of *Dactylis glomerata s.l.* In: *FUNCTIONAL ECOLOGY* 13. 473–482.

Salinger, M. (2005): Climate variability and change: Past, present and future - An overview. In: *CLIMATIC CHANGE* 70. 9–29.

Savolainen, O.; Pyhajarvi, T.; Knurr, T. (2007): Gene flow and local adaptation in trees. In: *ANNUAL REVIEW OF ECOLOGY EVOLUTION AND SYSTEMATICS* 38. 595–619.

Schaberg, P.G.; DeHayes, D.H.; Hawley, G.J.; Nijensohn, S.E. (2008): Anthropogenic alterations of genetic diversity within tree populations: Implications for forest ecosystem resilience. In: *FOREST ECOLOGY AND MANAGEMENT* 256. 855–862.

Schar, C.; Jendritzky, G. (2004): Climate change: Hot news from summer 2003. In: *NATURE* 432. 559–560.

Scharnweber, T.; Manthey, M.; Criegee, C.; Bauwe, A.; Schroeder, C.; Wilmking, M. (2011): Drought matters - Declining precipitation influences growth of *Fagus sylvatica L.* and *Quercus robur L.* in north-eastern Germany. In: *FOREST ECOLOGY AND MANAGEMENT* 262. 947–961.

Scherrer, D.; Bader, M.; Koerner, C. (2011): Drought-sensitivity ranking of deciduous tree species based on thermal imaging of forest canopies. In: *AGRICULTURAL AND FOREST METEOROLOGY* 151. 1632–1640.

Schönwiese, C.-D., Staeger, D, Trömel, S. (2005): Klimawandel und Extremereignisse in Deutschland. Klimastatusbericht 2005, Deutscher Wetterdienst.

Schraml, C.; Rennenberg, H. (2000): Sensitivity of different ecotypes of beech trees (*Fagus sylvatica L.*) to drought stress. In: *FORSTWISSENSCHAFTLICHES CENTRALBLATT* 119. 51–61.

Seho, M.; Kohnle, U.; Albrecht, A.; Lenk, E. (2010): Growth analyses of four provenances of European Black Pine (*Pinus nigra*) growing on dry sites in southwest Germany (Baden-Wuerttemberg). In: *ALLGEMEINE FORST UND JAGDZEITUNG* 181. 104–116.

Signarbieux, C.; Feller, U. (2012): Effects of an extended drought period on physiological properties of grassland species in the field. In: *JOURNAL OF PLANT RESEARCH* 125. 251–261.

Signarbieux, C.; Feller, U. (2011): Non-stomatal limitations of photosynthesis in grassland species under artificial drought in the field. In: *ENVIRONMENTAL AND EXPERIMENTAL BOTANY 71*. 192–197.

Smith, J.B.; Schneider, S.H.; Oppenheimer, M.; Yohe, G.W.; Hare, W.; Mastrandrea, M.D. et al. (2009): Assessing dangerous climate change through an update of the Intergovernmental Panel on Climate Change (IPCC) “reasons for concern". In: *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* 106. 4133–4137.

Smith, M.D. (2011): The ecological role of climate extremes: current understanding and future prospects. In: *JOURNAL OF ECOLOGY* 99. 651-655.

Solomon, S.D.; Qin M.; Manning Z.; Chen M.; Marquis K.B; Averyt M. and H.L Miller (Hg.) (2007): Climate Change 2007. The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC. Cambridge: University Press Cambridge.

Soto, A.; Robledo-Arnuncio, J.J.; Gonzalez-Martinez, S.C.; Smouse, P.E.; Alia, R. (2010): Climatic niche and neutral genetic diversity of the six Iberian pine species: a retrospective and prospective view. In: *MOLECULAR ECOLOGY* 19. 1396–1409.

Sowerby, A.; Emmett, B.A.; Tietema, A.; Beier, C. (2008): Contrasting effects of repeated summer drought on soil carbon efflux in hydric and mesic heathland soils. In: *GLOBAL CHANGE BIOLOGY* 14. 2388–2404.

Thomas, C.D.; Cameron, A.; Green, R.E.; Bakkenes, M.; Beaumont, L.J.; Collingham, Y.C. et al. (2004): Extinction risk from climate change. In: *NATURE* 427. 145–148.

Thuiller, W.; Lavorel, S.; Araujo, M.; Sykes, M.; Prentice, I. (2005): Climate change threats to plant diversity in Europe. In: *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* 102. 8245-8250.

Trenberth, K.E. (2011a): Attribution of climate variations and trends to human influences and natural variability. In: *WILEY INTERDISCIPLINARY REVIEWS-CLIMATE CHANGE* 2 (6), S. 925–930.

Trenberth, K.E. (2011b): Changes in precipitation with climate change. In: *CLIMATE RESEARCH* 47. 123–138.

Unkasevic, M.; Tosic, I. (2011): The maximum temperatures and heat waves in Serbia during the summer of 2007. In: *CLIMATIC CHANGE* 108. 207–223.

van Peer, L.; Nijs, I.; Reheul, D.; Cauwer, B. de (2004): Species richness and susceptibility to heat and drought extremes in synthesized grassland ecosystems: compositional vs physiological effects. In: *FUNCTIONAL ECOLOGY* 18. 769–778.

Vanhanen, H.; Veleli, T.O.; Paivinen, S.; Kellomaki, S.; Niemela, P. (2007): Climate change and range shifts in two insect defoliators: Gypsy moth and nun moth - A model study. In: *SILVA FENNICA* 41. 621–638.

Varelides, C.; Brofas, G.; Varelides, Y. (2001): Provenance variation in *Pinus nigra* at three sites in Northern Greece. In: *ANNALS OF FOREST SCIENCE* 58. 893–900.

Von Wuehlisch, G.; Krusche, D.; Muhs, H. J. (1995): Variation in temperature sum requirement for flushing of beech provenances. In: *SILVAE GENETICA* 44. 343–346.

Vornam, B.; Decarli, N.; Gailing, O. (2004): Spatial distribution of genetic variation in a natural beech stand (*Fagus sylvatica L.*) based on microsatellite markers. In: *CONSERVATION GENETICS* 5. 561–570.

Walther, G.R.; Beissner, S.; Burga, C.A. (2005): Trends in the upward shift of alpine plants. In: *JOURNAL OF VEGETATION SCIENCE* 16. 541–548.

Walther, G. R.; Post, E.; Convey, P.; Menzel, A.; Parmesan, C.; Beebee, T. J.C. et al. (2002): Ecological responses to recent climate change. In: *NATURE* 416. 389–395.

Walther, G.R.; Roques, A.; Hulme, P.E.; Sykes, M.T.; Pysek, P.; Kuehn, I. et al. (2009): Alien species in a warmer world: risks and opportunities. In: *TRENDS IN ECOLOGY & EVOLUTION* 24. 686–693.

Weisshuhn, K.; Auge, H.; Prati, D. (2011): Geographic variation in the response to drought in nine grassland species. In: *BASIC AND APPLIED ECOLOGY* 12. 21–28.

Westgarth-Smith, A.R.; Leroy, S.A.G.; Collins, P.E.F.; Harrington, R. (2007): Temporal variations in English populations of a forest insect pest, the green spruce aphid (*Elatobium abietinum*), associated with the North Atlantic Oscillation and global warming. In: *QUATERNARY INTERNATIONAL* 173. 153–160.

Woldendorp, G.; Hill, Michael J.; Doran, R.; Ball, M.C. (2008): Frost in a future climate: modelling interactive effects of warmer temperatures and rising atmospheric [CO(2)] on the incidence and severity of frost damage in a temperate evergreen (*Eucalyptus pauciflora*). In: *GLOBAL CHANGE BIOLOGY* 14. 294–308.

Wolkovich, E.M.; Cook, B.I.; Allen, J.M.; Crimmins, T.M., Betancour, J.L. et al. (2012): Warming experiments underpredict plant phenological response on climate change. In: NATURE doi:10.1038/nature11014.

Wortemann, R.; Herbette, S.; Barigah, T.S.; Fumanal, B.; Alia, R.; Ducousso, A. et al. (2011): Genotypic variability and phenotypic plasticity of cavitation resistance in *Fagus sylvatica L.* across Europe. In: *TREE PHYSIOLOGY* 31. 1175–1182.

Yachi, Shigeo; Loreau, Michel (1999): Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. In: *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* 96. 1463–1468.

Zebisch, M.; Grothmann T.; Schröter D.; Hasse C.; Fritsch U.; Cramer W. (2005): Climate Change-Klimawandel in Deutschland. Vulnerabilität und Anpassungsstrategienklimasensitiver Systeme. Hg. v. Umweltbundesamt. Potsdam-Institut für Klimafolgenforschung (UBA Forschungsbericht).

Zhu, K.; Woodall, C.W.; Clark, J.S. (2012): Failure to migrate: lack of tree range expansion in response to climate change. In: *GLOBAL CHANGE BIOLOGY* 18. 1042-1052.

5. Declaration of Own Contribution

Concept: Idea for the study and development of experimental design or development of concepts for opinion article.

Data acquisition: Organization and execution of data acquisition measurements together with the help of technicians, students and interns.

Data analysis: Statistical analysis of data and illustration in tables and figures.

Writing: Writing the manuscripts, including literature research

Editing: Proof-reading and grammar editing, including comments and inputs from co-authors and their integration in the manuscript and preparation for resubmissions after the manuscript was reviewed by the journals referees.

Manuscript 1:

Restoration Ecology 19(4), 433-440 (2011)

Assisted colonization: a question of focal units and recipient localities

Juergen Kreyling, Torsten Bittner, Anja Jaeschke, Anke Jentsch, Manuel Jonas Steinbauer, **Daniel Thiel**, Carl Beierkuhnlein

Concept: 10 %

Data acquisition: (review article without data)

Data analysis: -

Writing: 0 %

Editing: 10 %

Manuskript 2:

Forest Ecology and Management 270, 200-208 (2012)

Uniform drought and warming responses in *Pinus nigra* provenances despite specific overall performances

Daniel Thiel, Laura Nagy, Carl Beierkuhnlein, Gerhard Huber, Anke Jentsch, Monika Konnert, Juergen Kreyling

Concept: 50 %

Data acquisition: 90 %

Data analysis: 90 %

Writing: 100 %

Editing: 50 %

Manuskript 3:

European Journal of Forest Research (in press)

Different reactions of central and marginal provenances of Fagus sylvatica to experimental drought

Daniel Thiel, Laura Nagy, Carl Beierkuhnlein, Kolja Egen, Gerhard Huber, Anke Jentsch, Monika Konnert, Juergen Kreyling, Constanze Buhk

Concept: 25 %

Data acquisition: 10 %

Data analysis: 100 %

Writing: 100 %

Editing: 25 %

Manuskript 4:

Journal of Ecology 99, 703-713 (2011)

SPECIAL FEATURE: ECOLOGICAL CONSEQUENCES OF CLIMATE EXTREMES

Ecotypes of European grass species respond differently to warming and extreme drought

Carl Beierkuhnlein, **Daniel Thiel**, Anke Jentsch, Evelin Willner, Juergen Kreyling

Concept: 25 %

Data acquisition: 100 %

Data analysis: 75 %

Writing: 25 %

Editing: 25 %

Manuscript 5:

Environmental and Experimental Botany 78, 99-108 (2012)

Cold hardiness of *Pinus nigra* Arnold as influenced by geographic origin, warming, and extreme summer drought

Juergen Kreyling, Guido L.B. Wiesenberg, Daniel Thiel, Christian Wohlfart, Gerhard Huber, Julia Walter, Anke Jentsch, Monika Konnert, Carl Beierkuhnlein

Concept: 10%

Data acquisition: 25 %

Data analysis: 10%

Writing: 10%

Editing: 10%

Manuscript 6:

European Journal of Forest Research 131(3), 717-725 (2012)

Late frost sensitivity of juvenile *Fagus sylvatica* L. differs between southern Germany and Bulgaria and depends on preceding air temperature

Jürgen Kreyling , Daniel Thiel , Laura Nagy, Anke Jentsch, Gerhard Huber, Monika Konnert, Carl Beierkuhnlein

Concept: 25%

Data acquisition: 50 %

Data analysis: 25 %

Writing: 10 %

Editing: 25 %

Manuscript 7:

Ecography, 35(3), 268-275 (2012)

Ecotypic differentiation and past climatic experience influence the response to late spring frost in four common grass species in Central Europe

Juergen Kreyling, Daniel Thiel, Katrin Simmnacher, Evelin Willner, Anke Jentsch, Carl Beierkuhnlein

Concept: 25 %

Data acquisition: 50 %

Data analysis: 25 %

Writing: 10 %

Editing: 25 %

Manuscript 8:

Journal

Intraspecific diversity abets chances of high yields under different climatic conditions – a modelled example from provenance trial data

Daniel Thiel, Klara Dolos, Carl Beierkuhnlein, Anke Jentsch, Björn Reineking, Camilla Wellstein, Jürgen Kreyling

Concept: 25%

Data acquisition: 100%

Data analysis: 25%

Writing: 75%

Editing: 50%

6. Manuscripts

6.1. Manuscript 1:

Assisted colonization: a question of focal units and recipient localities

Restoration Ecology 19(4), 433-440 (2011)

Juergen Kreyling[a], Torsten Bittner[a], Anja Jaeschke[a], Anke Jentsch[b], Manuel Jonas Steinbauer[a], Daniel Thiel[a], Carl Beierkuhnlein[a]

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Keywords

adaptation strategy, assisted migration, core species, ecosystem engineers, managed relocation, managed translocation, species conservation

Abstract

Assisted colonization as an adaptation strategy to conserve or restore biodiversity in the face of climate change deservedly evokes controversy. Assisted colonization is perceived by some as a last option for conserving endangered species and by others as a risky and unwise management effort due to current gaps of knowledge. Based on the pros and cons of the recent debate, we show that the current discussion mainly focuses on the assisted colonization of rare and endangered species beyond their natural range of distribution. We suggest that a more useful approach for the conservation of endangered species could occur by focusing on the relevant foundation or keystone species, which ensure ecosystem integrity for a multitude of dependent species by governing the habitat structure and micro-climate of the site. Examples of foundation species include dominant tree species in forests or dominant corals in coral reefs. For a given conservation or restoration need (e.g. conservation of rare species), we recommend the assisted colonization of pre-adapted ecotypes of the relevant foundation species from climates similar to future expectations for the target site. This approach could lead to climate-safe habitats for endangered species with minimal adverse effects on recipient ecosystems.

Introduction

Assisted colonization as a conservation tool against the adverse effects of climate change on biodiversity is currently receiving a lot of attention (see Hoegh-Guldberg et al. 2008 or Ricciardi & Simberloff 2009 and the various replies to these articles). This debate is based on the notion that global climate change leads to high regional extinction risks for species' populations (Thomas et al. 2004). Naturally rare species and species already exposed to habitat decline are assumed to be especially threatened by climate change (Ohlemüller et al. 2008). Yet, the degree to which such species are limited directly by climate or by biotic (Pelini et al. 2009) or other environmental factors (Harsch et al. 2009) remains largely unexplored. As a consequence of limited information, advocates and opponents of assisted colonization differ in their perception of the benefits and risks of this approach. Although, it is usually regarded as a final option for conservation (e.g. Hoegh-Guldberg et al. 2008), assisted colonization has to be considered as one management option if preventing climate-driven extinction is a conservation priority (McLachlan et al. 2007; Richardson et al. 2009).

We define assisted colonization as 'the intentional movement of focal units (ecotypes, species, taxa, functional types, life forms) to recipient localities, where these focal units are currently absent, and where they cannot be expected to colonize by natural means within a short time frame (i.e. years or decades)'. We agree with Hunter (2007) that assisted colonization is a more appropriate term than assisted migration, because the final goal of this approach is not only to assist dispersal but rather the successful establishment of individuals and the subsequent development of self-sustaining populations, which is much more demanding. 'Managed relocation' or 'managed translocation' are further used as synonyms for this approach. However, these terms are not necessarily confined to the context of adaptation against climate change (Ricciardi & Simberloff 2009).

Evaluation of the applicability of assisted colonization needs to focus on the impacts of conducting (or not conducting) assisted colonization on a given focal unit and the impacts of assisted colonization on the recipient ecosystem (including aspects such as novel biotic interactions with other taxa, rapid genetic evolution at the recipient locality, changes in niche space within the recipient ecosystem, functional redundancy or complementarity to other species in the recipient community). Furthermore, the practical feasibility of conducting assisted colonization and the societal acceptability need to be considered (Richardson et al. 2009).

Assisted colonization is already taking place in various instances and therefore scientifically sound recommendations are urgently needed. Commercial nurseries in Europe, for example,

have successfully assisted the colonization of at least 260 native European plant species by ornamental planting at an average of 1000 km north of the species' natural northern range limits (van der Veken et al. 2008). During the early 20th century various cases of assisted colonization of plant species occurred in Central Europe. Their aim was to establish mainly thermophilous species in special microhabitats (e.g. steep south facing slopes) in order to enrich regional floras (e.g. *Iris variegata*, *Linum austriacum*, *Verbascum phoeniceum*). Several of these species are still present with stable populations (Walter 2003). Here, it should be noted that Pleistocene history provides evidence of the importance of localities differing in their microclimate from the predominant macroclimate of a region for species survival (Bush 2002). In forestry, there is an extensive history of successful and unsuccessful assisted colonization trials (Zobel et al. 1987). Foresters move genetic resources from their point of origin to locations beyond their natural dispersal ranges in particular during regeneration after timber harvesting. Here, managers are already making important decisions about the climatic tolerance and transferability of tree seeds (McKenney et al. 2009). In Canada, legislative recommendations concerning assisted colonization as a management tool to address climate change are already in place for British Columbia (O'Neill et al. 2008).

Here, we shortly present the arguments for and against assisted colonization. We then discuss the proposed schemes for the selection of focal units and recipient localities. We suggest focusing on local adaptations of foundation or keystone species (Ellison et al. 2005) and moving them within the species range in order to produce climate-safe habitats upon which endangered or rare species depend, rather than directly relocating endangered species. This approach provides a new dimension to the recent debate on assisted colonization and might help to reach a consensus between supporters and opponents of assisted colonization because it can be applied with minimal risks, especially in many restoration efforts.

Arguments in favor of assisted colonization

The paramount argument for the intentional movement of focal units is to mitigate elevated extinction risk due to climate change (Thomas et al. 2004). The underlying assumption is that focal units lack adaptation and dispersal abilities to cope with rapid climate change. Other arguments that support assisted colonization (Table 1) are generally subordinate. Several points merely aim at defending the overall approach, such as the notion that low risk situations can be identified and that a movement of species is generally not accompanied by any detrimental effects in the majority of studied cases. All of these considerations are based on the assumption that the risk of no action would be greater for biodiversity than the risks

involved in assisted colonization. Most authors focus on the species level, but the maintenance of genetic diversity within certain species is also recognized as a goal of assisted colonization (del Castillo et al. 2009).

A different view prevails in forestry, where economic benefits (i.e. a high and stable productivity of ecosystems) are given top priority (Chapin et al. 2007; McKenney et al. 2009). This reasoning shifts the focus of attention from taxonomic units to the stability and productivity of a given geographic unit. The discussion about assisted colonization among conservationists might benefit from an acknowledgement of this view, as the creation of habitats adapted to climate change might meet several conservation goals.

Table 1: Arguments for assisted colonization.

The Pros	References (not comprehensive)
Reduced risk of extinction of focal units which lack adaptation and dispersal abilities to cope with climate change (intensified by fragmentation)	Davidson & Simkanin 2008; Hoegh-Guldberg et al. 2008; Hunter 2007; McLachlan et al. 2007; Mueller & Hellmann 2008; Pelini et al. 2009; Ricciardi & Simberloff 2009; Richardson et al. 2009; Willis et al. 2009
Conservation of genetic diversity	del Castillo et al. 2009
Identification of low risk situations possible	Hoegh-Guldberg et al. 2008; Willis et al. 2009
Risks generally low, as the majority of studied historical species introductions were not detrimental	Mueller & Hellmann 2008
Risk of adverse effects on biodiversity from inaction higher	Schwartz et al. 2009
Conservation and restoration of diverse forests in the face of climate change	Chapin et al. 2007
Maintenance of the productive potential and stability (of forests)	Chapin et al. 2007; McKenney et al. 2009
Active conservation as opposed to documentation of losses for conservationists	Ricciardi & Simberloff 2009
Technical feasibility (at least for some taxa)	van der Veken et al. 2008; Willis et al. 2009
Pragmatic and cost-effective approach	Willis et al. 2009

Arguments against assisted colonization, including open research questions

Intended and unintended species introductions can result in unpredictable consequences for the recipient ecosystems in terms of their nutrient cycling, productivity, disturbance regime, biotic interactions, and dispersal patterns (Ricciardi & Simberloff 2009). These notions serve as the main arguments against assisted colonization (Table 2). A primary focus of invasion biology over recent decades has been the search for predictors of the invasive potential of taxa. Thus far, these predictors have remained elusive (Levine et al. 2003) although some trait based risk assessments have been made (e.g. Reichard & Hamilton 1997 for woody plants or Kolar & Lodge 2002 for fish). It has to be stressed, however, that the majority of unintended introductions do not lead to successful establishment of populations and only very few of those that establish do become invasive (Richardson & Pysek 2006).

Table 2: Arguments against assisted colonization.

The cons	References (not comprehensive)
Risk of adverse effects on native species composition and ecosystem functioning (as shown by examples of invasive species)	Chapin et al. 2007; Davidson & Simkanin 2008; Hoegh-Guldberg et al. 2008; Hunter 2007; McLachlan et al. 2007; Mueller & Hellmann 2008; Ricciardi & Simberloff 2009; Richardson et al. 2009; van der Veken et al. 2008; Willis et al. 2009
High risks for recipient ecosystems even for short distance translocations	Davidson & Simkanin 2008; Mueller & Hellmann 2008; Ricciardi & Simberloff 2009
Single species approach untimely in conservation	Davidson & Simkanin 2008
Ex-situ conservation more effective	Davidson & Simkanin 2008
Impediment of efforts to preserve or restore habitats	Hunter 2007; Ricciardi & Simberloff 2009; Richardson et al. 2009
Problematic identification of recipient localities with imperfect knowledge on ecology and climate change	del Castillo et al. 2009; Hunter 2007; McLachlan et al. 2007
Technical feasibility generally questionable	Pelini et al. 2009; Ricciardi & Simberloff 2009
Technical feasibility for rare and endangered species not provided	Hunter 2007; Mueller & Hellmann 2008
Lack of predictive methods for risk assessment	Ricciardi & Simberloff 2009
Some current climate conditions are without future definition, assisted colonization for species from these climates is not feasible	Williams et al. 2007
Unknown costs and acceptability	Richardson et al. 2009
Creation of a biased flora or fauna	Schwartz et al. 2009
Biological homogenization	van der Veken et al. 2008

An important research question with regard to assisted colonization is whether the target species are directly limited by specific climatic conditions. A direct dependence of species on current macro-climate is questionable for many species (Pearson & Dawson 2003; Guisan & Thuiller 2005). Even current patterns of ectotherm distributions are far from equilibrium with current macro-climate (Araujo & Pearson 2005). The biotic environment with its associated micro-climates may be of higher relevance to many rare species than macro-climatic conditions (Ellison et al. 2005; Harsch et al. 2009; Pelini et al. 2009).

Another contribution to uncertainty is the limited knowledge on effective dispersal rates (Clark et al. 2003; Higgins et al. 2003). Rarity and stochasticity inherent in long distance

dispersal strongly limit our ability to forecast the spread of focal units (Nathan et al. 2008). Finally, recent findings indicate that environmental change, including climate change, can even cause rapid phenotypic change through both ecological and evolutionary processes especially in small populations (Ozgul et al. 2009). Common species, on the other hand, generally possess high genetic variability which allow for adaptation via selection (Bischoff et al. 2010). Species reproducing primarily through vegetative means, however, may be at a disadvantage. Such kind of biotic adaptations are neglected in scenarios of biodiversity loss.

Focal units – what should be moved?

Rare and endangered species are challenged most by climate change and are therefore usually discussed as focal units for assisted colonization. We see, however, three reasons why rare and endangered species are not well suited for assisted colonization efforts:

(1) For many rare and endangered species no adequate recipient locality can be found. Current centers of rare species distributions are located in areas with highly specific climate conditions, which are predicted to shrink disproportionately under future climate change (Ohlemüller et al. 2008) or even be lost globally (Williams et al. 2007). Novel conditions may further be produced by interactions among climate, local static environmental conditions (e.g., soils), and local species compositions.

(2) The collection of sufficient numbers of individuals for establishment needs is hardly possible without harming local populations of endangered species. Willis et al. (2009) used two butterfly species and demonstrated the short-term (6 years) feasibility of assisted colonization in a field experiment. They worked with common species and translocated 500 and 600 individuals of the two species respectively. Based on a meta-analysis of published studies, Traill et al. (2007) suggest a minimum of 1,650 – 100,000 individuals for viable population sizes of insects in order to gain a persistence probability of 99% within a time frame of 40 generations. Collecting such numbers would cause extirpations of source populations without the insurance of successful colonization at the new locality.

(3) Declining populations may have already passed through a genetic bottleneck and assisted colonization is doomed to fail in such cases. Remnant populations (Eriksson 1996) represent only a limited part of the previous gene pool. An excess extinction risk of naturally small populations of butterflies, for instance, can be related to inbreeding depression (Saccheri et al. 1998). The history of reintroduction projects (Mueller & Hellmann 2008) implies that the risk of failed translocations is considerably higher for rare species (54% failing) than for common species (only 14% failings) (Griffith et al. 1989, Wolf et al. 1996). Such failings are not only

detrimental to the translocated individuals, but also to the source populations due to its depletion of individuals.

Based on these concerns and taking also the decision tree provided by Hoegh-Guldberg et al. (2008) into account, assisted colonization might only be an option for a very limited number of endangered species with sufficiently large gene pools and well-known climatic and ecological constraints that can be met in new target localities. Those species most threatened by climate change would not appear to be suitable in most cases. The problem of a global loss of rare species cannot be addressed by this means. We conclude that assisted colonization of rare and endangered species themselves is risky, ineffective and probably hazardous for both the focal units as well as for the recipient ecosystems in many cases.

The creation and conservation of climate-safe habitats, i.e. habitats that can be expected to withstand climate change without changes to their overall structure and functionality, may be more profitable in many cases. We already discussed that a direct dependence of species on current macro climate appears questionable for many species. Fine scale distributions can further be expected to depend strongly on micro-climatic variation within a landscape. Temperature variation due to exposition and vegetation cover within the same region and elevation is stronger than the projected increases in mean temperature until the end of this century (Scherrer and Korner 2010). Based on these considerations, rare species might be conserved without the need to move them by adapting their habitats. Such climate-safe habitats depend mainly on the climatic tolerance of the dominant plants, which determine structure and micro-climate. According to Ellison et al. (2005), such species could be called core species, keystone species, structural species, ecosystem engineers or, as further used here, foundation species, i.e. “single species that define much of the structure of a community by creating locally stable conditions for other species, and by modulating and stabilizing fundamental ecosystem processes”.

Forestry has a long tradition in assisted colonization of foundation species (Zobel et al. 1987; Chapin et al. 2007; McKenney et al. 2009). Although the main concern in forestry is not the preservation of biodiversity, forest trees provide specific environments that serve as habitats for entire communities of plants, animals, and microorganisms. Ensuring the presence of these species supplies climate-safe habitats for a multitude of dependent species. For instance, several thousand species, such as plants, insects or fungi, depend on the European beech (*Fagus sylvatica*) as a foundation species (Kölling et al. 2005). The loss of such foundation species is expected to have cascading, adverse effects on biodiversity and ecosystem functioning. Replacing native species by exotic ones may safeguard biomass production in

forestry, but would negatively affect conservation value. Furthermore, pollinator systems provide ample examples of rare species performance, sometimes even survival, depending on the presence of common species (e.g. Gibson et al. 2006). Coral species (Acroporidae) with wide ranges may serve as another example here. In corals, low-latitude populations exist which show higher temperature tolerances than those at higher latitudes (Berkelmans & van Oppen 2006). The latter have already declined or died off due to increased thermal stress. Introducing lower-latitude, heat-adapted ecotypes to these degraded sites may therefore serve as a useful management strategy (Berkelmans & van Oppen 2006). These examples and several related reviews (e.g. Simberloff 1998; Boogert et al. 2006) suggest that foundation species and their relationships with biodiversity are common phenomena. The main question is therefore how to retain or restore the foundation species in times of change.

Moving foundation species out of their natural range (i.e., the potential current range in the absence of human interference), can be expected to create even larger ecological problems (e.g., invasiveness) than moving rare species due to the generally higher competitive power of foundation species (Hunter 2007). Foundation species are commonly wide ranging species (Ellison et al. 2005) that exhibit large genetic variation (Hamrick 2004). This is commonly displayed in strong local adaptation, especially to the climate (Joshi et al. 2001, McKay et al. 2005). Ecotypes can be found within the current distribution of the foundation species that are adapted to future climate conditions at locations further north or at higher altitudes (Figure 1). Moving such pre-adapted genetic resources to sites where the species is already present or where it was present before human interference in case of sites subjected to restoration efforts would ensure ecosystem integrity by conserving the presence of foundation species and by providing climate-safe habitats for a host of dependant species. Within-species range translocation might also minimize potential problems with invasiveness (but see below).

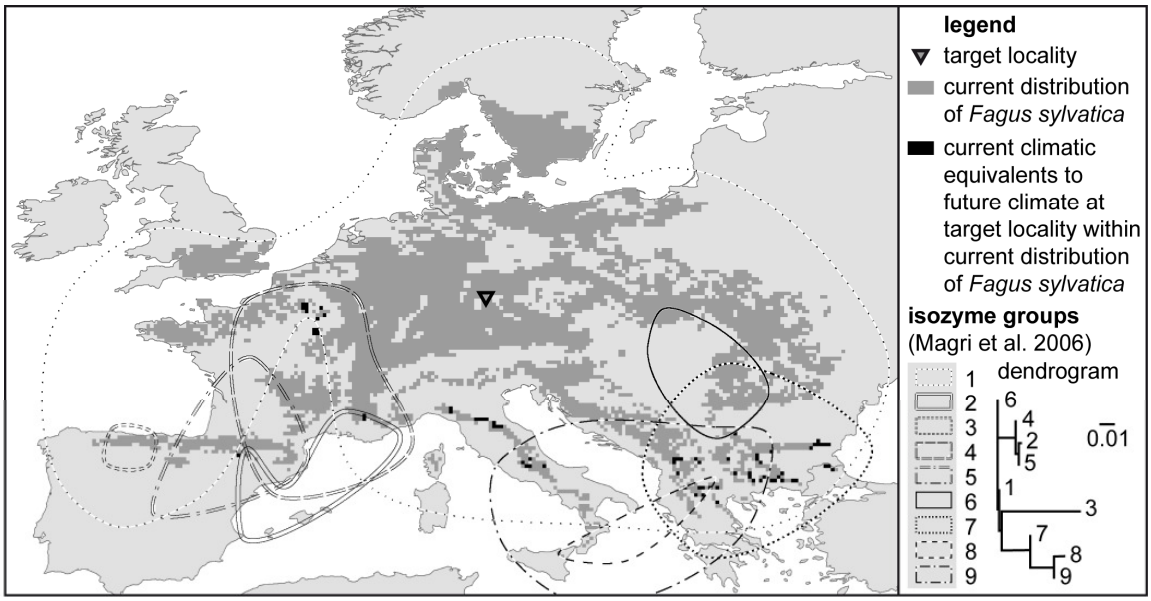

Figure 1: The proposed assisted ecotype colonization of foundation species exemplified for a given site at Bayreuth, Germany (triangle) where European beech (*Fagus sylvatica*) is the foundation species for the maintenance of (semi-) natural forests which contain a high number of specialiced species (Kölling et al. 2005). Its current distribution according to EUFORGEN (2009) is shown in dark grey. Current climate equivalents for the target site at the end of this century (regional climate model: REMO-BfG (MPI-M Hamburg); SRES: A1B) based on mean annual temperature +/- 0.5°C, colder mean winter temperature, and lower summer precipitation are shown in black (current climate based on worldclim; Hijmnans et al. 2005). Outlined are different genotypes of the target species (based on isozyme similarity; Magri et al. 2006) which imply that pre-adapted ecotypes of different genetic heritage would be available.

Genetic diversity of target species increases restoration success (Bischoff et al. 2010). It has also been proposed that a wider selection of "mixtures of genotypes from climatically local populations" might benefit short-term establishment and long-term adaptation potential (McKay et al. 2005). We suggest moving one step further by adding genetic resources from ecotypes of current climatic conditions comparable to the expected future climate of the target area (Figure 1). Including this approach into general restoration concepts seems to be a promising scheme for the ecological adaptation of landscapes to climate change, which is an inevitable challenge for any restoration effort nowadays. Based on all arguments made above and on the associated uncertainties, the assisted colonization of pre-adapted ecotypes may more often be a successful management strategy than the assisted colonization of rare and endangered species (Figure 2).

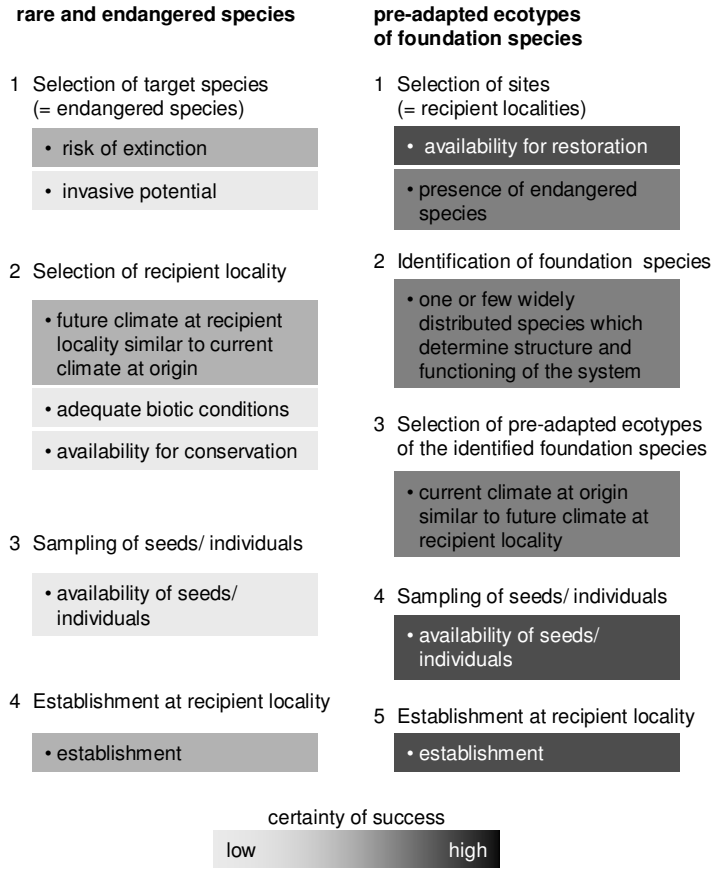

Figure 2: Conceptual comparison of the basic steps in assisted colonization for different focal units. The "pre-adapted ecotypes" approach starts from a given location which either is available for restoration or contains an endangered species and aims at the climate-safe adaptation of this given location. The expected certainty of success for each step is based on subjective expert knowledge by the authors.

This assisted ecotype colonization of foundation species does not come without risks. Two major aspects of genetic pollution, i.e. the introduction of non-native genetic information, have to be considered according to McKay et al. (2005). The first concern is that the introduction of nonlocal genotypes will create a large genetic load that causes the restoration to fail. To counter this, we strongly advise to test the suitability of target ecotypes experimentally beforehand and to use origins from matching climatic and environmental conditions (Figure 1). The usage of more than one origin appears to be a further insurance for success. The second concern is that the nonlocal genotypes will eliminate locally adapted genotypes. Here, we argue that a high percentage of restoration efforts should be carried out after the target species is already lost from the site and local adaptation, consequently, is lost as well. If the target species is still present, any superiority of local adaptation should also result in better fitness and (re-) selection of the best adapted genotypes.

The consequences of an assisted colonization of pre-adapted ecotypes within the range of the target foundation species should be predictable. No adverse effects on the recipient system comparable to the expected major risks of moving species outside of their natural range and even outside of their biogeographical context are expected simply because the species is or was already present, even dominant, in the system before the action (Figure 2). Figure 1 displays an example of the proposed approach. In accordance with Hunter (2007), we strongly warn against the assisted colonization of foundation species outside their biogeographical contexts.

There are further caveats to the idea of assisted colonization in general, as it might not suffice to select recipient localities based on the similarity of their expected future average climate conditions (i.e. mean temperature and precipitation sum) with the climate of the focal units' origin. Extreme weather events might put much higher selective pressure on survival than average climate conditions while hardly being quantifiable at a sufficient spatial resolution both in the past and in future expectations (Jentsch & Beierkuhnlein 2008). Experimental testing of the tolerance of target genotypes in the face of expected extreme events is, therefore, recommended.

Furthermore, the problem of targeting recipient localities with suitable climate conditions is a question of the appropriate time scale, as the recipient localities themselves are also subject to a changing climate. McKenney et al. (2009) suggest that the selection of recipient localities in forestry should depend on the silvicultural rotation lengths of the focal units, with climate conditions similar enough to the climate preferences of the focal units to enable a good survival rate while also ensuring good adaptation towards the end of the rotation. Comparable suggestions for the conservation of endangered species are missing. It is therefore important to explore how far species' distributions lag behind the shifting climatic conditions, especially in the anthropogenically modified landscape. Placing pre-adapted ecotypes of species at the leading edge of their current distribution may be the most effective (and conservative) intervention.

Conclusions

The debate about assisted colonization as a management tool to conserve biodiversity in the face of climate change is based largely on two opposing arguments. On the one hand, high extinction risks are projected due to the fact that focal units might not adapt fast enough to climate change through migration or genetic adaptation. Invasion biology, on the other hand, provides evidence that the intentional introduction of species may adversely affect recipient

ecosystems. A more important consideration may be that assisted colonization will not be feasible for many endangered taxa due to their limited availability and due to missing recipient localities. We conclude that the assisted colonization of single endangered species is risky and not useful in many cases.

Therefore, we propose that the structure and micro-climate of habitats containing rare and endangered species could be the focus for climate change adaptation (i.e. climate-safe habitats) via the establishment of pre-adapted ecotypes of the relevant foundation species. We suggest moving ecotypes of foundation species within the species' natural range in order to minimize possible adverse effects. The assisted colonization of foundation species ecotypes might provide for a means for sustainable climate change adaptation in restoration efforts. The history of conservation biology informs us that the restoration or conservation of suitable habitat including its dynamic processes (e.g. disturbance regimes) is the only sustainable option to manage endangered species. Building climate-safe habitats by the use of pre-adapted ecotypes of foundation species may therefore be a better investment toward the conservation of biodiversity than aiming at single endangered species.

Examples from forestry show that this approach is applicable with regard to societal or legislative dimensions. We see a strong need for future research on the role of foundation species, and on biotic interactions for ecosystem functioning in changing climates. Ultimately, the human dimension of assisted colonization, e.g. species selection, societal acceptance, legislative frameworks and costs require detailed consideration even if a consensus on ecological questions can be achieved.

Implications for practice

- Assisted colonization of rare and endangered species is risky for the target species and the recipient localities and cannot be recommended in general.
- Some endangered species could be conserved on site by developing climate-safe habitats through the assisted colonization of pre-adapted ecotypes of the relevant foundation species (e.g. major forest trees or corals). This approach would conserve structure and micro-climate of the habitat and avoid moving species out of their natural range.
- Regardless of the conservation approach, restoration efforts need to design climate-safe communities. The integration of ecotypes adapted to the expected future climate should become one part of restoration concepts.

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Literature cited

Araujo, M.B., and R.G. Pearson. 2005. Equilibrium of species' distributions with climate. Ecography **28**: 693–695.

Berkelmans, R., and M.J.H. van Oppen. 2006. The role of zooxanthellae in the thermal tolerance of corals: a `nugget of hope' for coral reefs in an era of climate change. Proceedings of the Royal Society of London Series B Biological Sciences **273**: 2305–2312.

Bischoff, A., T. Steinger, and H. Mueller-Schaerer. 2010. The importance of plant provenance and genotypic diversity of seed material used for ecological restoration. Restoration Ecology **18**: 338–348.

Boogert, N.J., D.M. Paterson, and K.N. Laland. 2006. The implications of niche construction and ecosystem engineering for conservation biology. Bioscience **56**: 570–578.

Bush, M.B. 2002. Distributional change and conservation on the Andean flank: a palaeoecological perspective. Global Ecology and Biogeography **11**: 463–473.

Chapin, I.F.S., K. Danell, T. Elmqvist, C. Folke, and N. Fresco. 2007. Managing climate change impacts to enhance the resilience and sustainability of Fennoscandian forests. Ambio **36**: 528–533.

Clark, J.S., M. Lewis, J.S. McLachlan, and J. HilleRisLambers. 2003. Estimating population spread: What can we forecast and how well? Ecology **84**: 1979–1988.

Davidson, I., and C. Simkanin. 2008. Skeptical of assisted colonization. Science **322**: 1048b-1049.

del Castillo, R.F., S. Trujillo Argueta, and C. Saenz-Romero. 2009. *Pinus chiapensis*, a keystone species: Genetics, ecology, and conservation. Forest Ecology and Management **257**: 2201–2208.

Ellison, A.M., M.S. Bank, B.D. Clinton, E.A. Colburn, K. Elliott, C.R. Ford, D.R. Foster, B. D. Kloeppel, J.D. Knoepp, G.M. Lovett, J. Mohan, D.A. Orwig, N.L. Rodenhouse, W.V. Sobczak, K.A. Stinson, J.K. Stone, C.M. Swan, J.Thompson, B. Von Holle, and J.R. Webster. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment **3**: 479–486.

Eriksson, O. 1996. Regional dynamics of plants: A review of evidence for remnant, source-sink and metapopulations. Oikos **77**: 248–258.

EUFORGEN. 2009. Distribution map of Beech (*Fagus sylvatica*). www.euforgen.org.

Gibson, R.H., I.L. Nelson, G.W. Hopkins, B.J. Hamlett, and J. Memmott. 2006. Pollinator webs, plant communities and the conservation of rare plants: arable weeds as a case study. Journal of Applied Ecology **43**: 246–257.

Griffith, B., J.M. Scott, J.W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool - status and strategy. Science **245**: 477–480.

Guisan, A., and W. Thuiller. 2005. Predicting species distribution: offering more than simple habitat models. Ecology Letters **8**: 993–1009.

Hamrick, J.L. 2004. Response of forest trees to global environmental changes. Forest Ecology and Management **197**: 323–335.

Harsch, M.A., P.E. Hulme, M.S. McGlone, and R.P. Duncan. 2009. Are treelines advancing? A global meta-analysis of treeline response to climate warming. Ecology Letters **12**: 1040-1049.

Higgins, S.I., J.S. Clark, R. Nathan, T. Hovestadt, F. Schurr, J.M. Fragoso, Aguiar, E. Ribbens, and S. Lavorel. 2003. Forecasting plant migration rates: managing uncertainty for risk assessment. Journal of Ecology **91**: 341–347.

Hijmans, R.J., S.E. Cameron, J.L. Parra, P.G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology **25**: 1965–1978.

Hoegh-Guldberg, O., L. Hughes, S. McIntyre, D.B. Lindenmayer, C. Parmesan, H.P. Possingham, and C.D. Thomas. 2008. Assisted colonization and rapid climate change. Science **321**: 345–346.

Hunter, M.L. 2007. Climate change and moving species: Furthering the debate on assisted colonization. Conservation Biology **21**: 1356–1358.

Jentsch, A., and C. Beierkuhnlein. 2008. Research frontiers in climate change: Effects of extreme meteorological events on ecosystems. Comptes Rendus Geoscience: **340**: 621–628.

Joshi, J., B. Schmid, M.C. Caldeira, P.G. Dimitrakopoulos, J. Good, R. Harris, A. Hector, K. Huss-Danell, A. Jumpponen, A. Minns, C. P. H. Mulder, J.S. Pereira, A. Prinz, M. Scherer-Lorenzen, A. S. D. Siamantziouras, A.C. Terry, A.Y. Troumbis, and J.H. Lawton. 2001. Local adaptation enhances performance of common plant species. Ecology Letters **4**: 536–544.

Kolar, C.S., and D.M. Lodge. 2002. Ecological predictions and risk assessment for alien fishes in North America. Science 298: 1233–1236.

Kölling, C., H. Walentowski, and H. Borchert. 2005. Die Buche in Mitteleuropa. AFZ - Der Wald **13**: 696–701.

Levine, J.M., M. Vila, C.M. D'Antonio, J.S. Dukes, K. Grigulis, and S. Lavorel. 2003. Mechanisms underlying the impacts of exotic plant invasions. Proceedings of the Royal Society London Series B Biological Sciences **270**: 775–781.

Magri, D., G.G. Vendramin, B. Comps, I. Dupanloup, T. Geburek, D. Gomory, M. Latalowa, T. Litt, L. Paule, J.M. Roure, I. Tantau, W. O. van der Knaap, R.J. Petit, and J.L. de Beaulieu. 2006. A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. New Phytologist **171**: 199–221.

McKay, J.K., C.E. Christian, S. Harrison, and K.J. Rice. 2005. "How local is local?" - A review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology* **13**: 432–440.

McKenney, D., J. Pedlar, and G. O'Neill. 2009. Climate change and forest seed zones: Past trends, future prospects and challenges to ponder. Forestry Chronicle **85**: 258–266.

McLachlan, J.S., J.J. Hellmann, and M.W. Schwartz. 2007. A framework for debate of assisted migration in an era of climate change. Conservation Biology **21**: 297–302.

Mueller, J.M.,and J.J. Hellmann. 2008. An assessment of invasion risk from assisted migration. Conservation Biology **22**: 562–567.

Nathan, R., F.M. Schurr, O. Spiegel, O. Steinitz, A. Trakhtenbrot, and A. Tsoar. 2008. Mechanisms of long-distance seed dispersal. Trends in Ecology and Evolution **23**: 638–647.

Ohlemüller, R., B.J. Anderson, M.B. Araujo, S.H.M. Butchart, O. Kudrna, R.S. Ridgely, and C.D. Thomas. 2008. The coincidence of climatic and species rarity: high risk to small-range species from climate change. Biology Letters **4**: 568–572.

O'Neill, G.A., N.K. Ukrainetz, M. Carlson, C.V. Cartwright, B.C. Jaquish, J.N. King, J. Krakowski, J.H. Russell, M.U. Stoehr, C.-Y. Xie, and A.D. Yanchuk. 2008. Assisted migration to address climate change in British Columbia - Recommendations for interim seed transfer standards. Ministry of Forests and Range, Forest Science Program, Tech Rep 048, Victoria, B.C.

Ozgul, A., S. Tuljapurkar, T.G. Benton, J.M. Pemberton, T.H. Clutton-Brock, and T. Coulson, 2009. The dynamics of phenotypic change and the shrinking sheep of St. Kilda. Science **325**: 464–467.

Pearson, R.G., and T.P. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Global Ecology and Biogeography **12**: 361–371.

Pelini, S.L., J.D.K. Dzurisin, K.M. Prior, C.M. Williams, T.D. Marsico, B.J. Sinclair, and J.J. Hellmann, 2009. Translocation experiments with butterflies reveal limits to enhancement of poleward populations under climate change. Proceedings of the National Academy of Sciences USA **106**: 11160–11165.

Reichard, S.H., and C.W. Hamilton. 1997. Predicting invasions of woody plants introduced into North America. Conservation Biology **11**: 193–203.

Ricciardi, A., and D. Simberloff. 2009. Assisted colonization is not a viable conservation strategy. Trends in Ecology and Evolution **24**: 248–253.

Richardson, D.M., J.J. Hellmann, J.S. McLachlan, D.F. Sax, M.W. Schwartz, P. Gonzalez, E.J. Brennan, A. Camacho, T.L. Root, O.E. Sala, S.H. Schneider, D.M. Ashe, J.R. Clark, R. Early, J.R. Etterson, E.D. Fielder, J.L. Gill, B.A. Minteer, S. Polasky, H.D. Safford, A.R. Thompson, and M. Vellend. 2009. Multidimensional evaluation of managed relocation. Proceedings of the National Academy of Sciences USA **106**: 9721–9724.

Richardson, D.M., and P. Pysek. 2006. Plant invasions: merging the concepts of species invasiveness and community invasibility. Progress in Physical Geography **30**: 409–431.

Saccheri, I., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature **392**: 491–494.

Scherrer, D., and C. Koerner. 2010. Infra-red thermometry of alpine landscapes challenges climatic warming projections. Global Change Biology **16**: 2602–2613.

Schwartz, M.W., J.J. Hellmann, J.S. McLachlan. 2009. The precautionary principle in managed relocation is misguided advice. Trends in Ecology and Evolution **24**: 474.

Simberloff, D. 1998. Flagships, umbrellas, and keystones: Is single-species management passé in the landscape era? Biological Conservation **83**: 247–257.

Thomas, C.D., A. Cameron, R.E. Green, M. Bakkenes, L.J. Beaumont, Y.C. Collingham, B. F. N. Erasmus, M. F. de Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A. S. van Jaarsveld, G.F. Midgley, L. Miles, M.A. Ortega-Huerta, A.T. Peterson, O.L. Phillips, and S.E. Williams. 2004. Extinction risk from climate change. Nature **427**: 145–148.

Traill, L.W., C.J.A. Bradshaw, and B.W. Brook. 2007. Minimum viable population size: A meta-analysis of 30 years of published estimates. Biological Conservation **139**: 159–166.

van der Veken, S., M. Hermy, M. Vellend, A. Knapen, K. Verheyen. 2008. Garden plants get a head start on climate change. Frontiers in Ecology and the Environment **6**: 212–216.

Walter, E. 2003. Florenverfälschung durch Ansalbung am Beispiel des NSG "Hohe und Zeyerner Wand" in Oberfranken. Bericht der Naturforschenden Gesellschaft Bamberg, **LXXVI**: 123–134.

Williams, J.W., S.T. Jackson, and J.E. Kutzbacht. 2007. Projected distributions of novel and disappearing climates by 2100 AD. Proceedings of the National Academy of Sciences USA **104**: 5738–5742.

Willis, S.G., J.K. Hill, C.D. Thomas, D.B. Roy, R. Fox, D.S. Blakeley, and B. Huntley. 2009. Assisted colonization in a changing climate: a test-study using two U.K. butterflies. Conservation Letters **2**: 46–52.

Wolf, C.M., B. Griffith, C. Reed, and S.A. Temple. 1996. Avian and mammalian translocations: Update and reanalysis of 1987 survey data. Conservation Biology **10**: 1142–1154.
Zobel, B.J., G. van Wyk, P. Stahl. 1987. Growing exotic forests. Wiley, New York, N.Y.

6.2. Manuscript 2:

Uniform drought and warming responses in Pinus nigra provenances despite specific overall performances

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Keywords

European Black Pine, local adaptation, within-species diversity, provenance trial, plant-climate interactions, lag effect

Abstract

Climate extremes are expected to increase in frequency and magnitude as a consequence of global warming, threatening the functioning, services and goods of forest ecosystems.

The introduction of species from drier and warmer climates is one option that is discussed to adapt forest ecosystems to these adverse effects of climate change. The (sub)-mediterranean *Pinus nigra* is a potential candidate for such assisted migration, especially for dry sites in Central Europe. The high genetic diversity within this species and thus the potential differences in adaptive capacity, however, makes it necessary to assess the response of *Pinus nigra* provenances to climatic extremes and identify suitable populations or ecotypes which are better adapted to projected future climates than indigenous species.

Here, we tested juvenile plants of 6 provenances of *Pinus nigra* for their response to different climate change scenarios (42-days drought and warming by 1.6K) in a full-factorial common-garden experiment in Bayreuth, Germany. In the second year only the warming treatment was imposed. Height, shoot quantity, mortality and needle phenology were determined for the two consecutive years.

Provenances differed in absolute growth (from 6.0 to 7.4 in 2009 and from 4.4 to 5.9cm in 2010) and survival rates (from 78.6% to 97.6%), but not in terms of shoot quantity and,

surprisingly, sensitivity to drought and warming. The drought treatment showed a delayed impact on height growth, as a significant growth reduction was detected for the second year (-2.6cm), but not for the first year, when the actual treatment took place. Drought decreases survival rates by 20.6%. The drought treatment had no effect on needle phenology.
Warming did not impact growth performance (height, shoot quantity). However, warming in combination with the drought treatment decreased the survival rate (-13%). Plants exposed to the warming treatment showed an earlier onset of needle development (-10.6 days).
Our results imply that no significant local adaptation of growth and survival to drought and warming occurs in *Pinus nigra.* Performance and growth response in face of warming and drought cannot be predicted by the climate at the places of origin. We therefore recommend that an introduction of *Pinus nigra* to regions outside its natural distribution range should not aim at introducing a single best-adapted provenance but at establishing populations with a high genetic diversity, e.g. by promoting natural regeneration in native stands or by intermixing with different genotypes in order to maintain a high adaptive capacity to climate change.

Introduction

In addition to the general warming trend, an increase in magnitude and frequency of extreme climatic events is expected during ongoing climate change (Min et al. 2011). This is especially relevant for long-living species and persistent ecosystems such as trees and forests. These can be strongly affected during their life cycle and successional trajectories and may react with declining performance or even breakdown. As a result, also the goods and services delivered by them are imperilled in face of increasing climatic extremes (Jentsch et al., 2007, Lindner et al., 2010, Allen et al., 2010).
Ecological responses to climatic extremes may be manifold (Jentsch & Beierkuhnlein, 2008). In a longer time scale, natural adaptation processes will take place at various levels ranging from molecular processes in the individual plant to the functioning of ecosystems. However, the pace of change is expected to exceed the adaptation capacity of current ecosystems. Depending on species and rate of change the inertia of established populations and the limited natural dispersal rates of trees may not allow direct responses to shifts in the climatic extreme events regime (Thomas et al., 2004, Lindner et al., 2010, Nathan et al., 2011). This mismatch has to be addressed by forest management.
In Central Europe, economically important coniferous species such as *Picea abies* are expected to suffer strongly under increased temperatures and reduced water availability in

summer (Maracchi et al. 2005). The cultivable acreage of this species may decrease dramatically (Kölling 2007; Kölling 2009). One possible adaptation strategy is the controlled introduction (or human assisted range shift) of tree species to sites out of their natural range. In the face of warming tendencies trees that are adapted to regionally projected warmer and drier conditions are taken into consideration (McKenney et al. 2009, Bolte et al. 2010, Kreyling et al. 2011b).

The European Black Pine (*Pinus nigra*) is a drought tolerant conifer (Isajev 2004, Huber 2011) with (sub)-Mediterranean distribution. Thus, it has been identified as one potential substitute for indigenous Central European conifers under future climatic conditions. Martinez-Vilalta et al. (2002) showed that mortality of *Pinus nigra* is significantly lower after two natural droughts than mortality of *Pinus sylvestris* in Eastern Spain. Saplings of *Pinus nigra* exhibit effective water stress regulation by stomatal control of transpirational water loss (Lebourgeois et al. 1998), which results in high drought tolerance. This adaptation strategy, however, is connected to reduced growth during water stress. Especially latewood formation seems to be especially sensitive to the amount of summer precipitation (Biel et al. 2004, Martin-Benito et al. 2008, Martin-Benito et al. 2010). Besides direct drought effects, delayed or lagged responses are documented. The climate, and especially precipitation of the previous year, impacts the growth in the early phase of the growing season (Lebourgeois 2000, Martin-Benito et al. 2008). Similar influences of precipitation and temperature conditions of the previous year on growth of *Pinus nigra, Pinus sylvestris* and *Pinus uncinata* are reflected by correlations between tree-ring chronologies and climatic records (Andreu et al. 2007). A strong influence of precipitation in the year prior to observed growing season on early wood formation is also reported for *Pinus sibirica* and *Larix decidua* (de Grandpre et al. 2011). Therefore, ther is a need to examine long-term impacts of climatic extreme events beyond current vegetation periods. However, all these studies report lagged drought response for mature trees. Juvenile stages may lack stored resources allowing for such a delayed response, yet, investigations on this aspect are lacking.

Dendrochronological data show that warming effects on the growth of *Pinus nigra* differ between geographic regions (Martin-Benito et al., 2010). In the temperate parts of its distribution, growth is stimulated by warming, whereas in the Mediterranean region warming increases water stress and thus has a negative influence on the growth of this species. Comparable results are reported for *Pinus contorta* in a Canadian provenance trial network (McLane et al. 2011). Experimental warming can furthermore shorten the time until drought-induced die-off of *Pinus edulis* trees (Adams et al. 2009).

Warming affects phenology, too, leading to earlier budburst in forest trees (e.g. Menzel et al. 1999). Especially species that have large ranges are likely to differ in phenology between geographical provenances, which has been shown in common garden experiments (Viveros-Viveros et al. 2009, Kandemir et al. 2010; Lu et al. 2011, Kreyling et al. 2011a). Therefore, the reciprocal control of provenance and global warming on phenological response has to be taken into account, when considering an assisted northward transfer of forest trees.

Phenotypic plasticity and the adaptive capacity of forest trees to climatic stressors, like drought, depend strongly on genetic composition and diversity (Hosius et al. 2006, Schaberg et al. 2008). *Pinus nigra* is known to exhibit a relatively high genetic diversity (Jagielska et al., 2007; Soto et al., 2010), with highest intra-provenance diversity in Serbian provenances (*ssp. nigra* ARNOLD) (Lucic et al. 2010) and lowest in Corsican provenances (*ssp. laricio* MAIRE), most probably due to pleistocene geographic isolation (Aguinagalde et al. 1997). Differences in genetic diversity within provenances or populations, most likely, display differences in adaptive capacity between provenances. Provenance trials revealed a diverse performance of provenances from various geographic origins (Varelides et al. 2001, Seho et al. 2010). Differences in germination rate within and among provenances in an experiment with five provenances and two habitat types of *Pinus nigra* suggest local adaptation to abiotic conditions, like soil type. (Mataruga et al. 2010). Furthermore, cold hardiness differs strongly in *Pinus nigra* provenances, with provenances from colder regions showing a higher level of cold hardiness (Kreyling et al. 2012).

Considering the outlined intraspecific variability in performance and the potential differences in the response to climate, it is important to identify whether certain provenances of *Pinus nigra* are better adapted to the expected future average climatic conditions and extremes. Such provenances might be potential candidates for the substitution of indigenous conifer species suffering under climate change.

Here, seedlings of six provenances of *Pinus nigra* ARNOLD were exposed to drought and warming in a full factorial common garden experiment over two years. We hypothesized that (1) provenances differ in terms of growth performance, mortality and phenological behaviour, and that (2) drought negatively impacts growth, increases mortality and delays needle phenology in general. We further expected that (3) warming has a positive impact on growth and survival, when there is no water shortage, and a negative impact under drought conditions due to increasing evapotranspiration and water stress. Finally, we assumed that (4) provenances exhibit local adaptations in their sensitivity to drought and warming.

Material and Methods

Experimental Site

The provenance-trial (EVENT 3) was established in March 2009 in Bayreuth, Germany, in the Ecological-Botanical Gardens of the University of Bayreuth. It is part of the EVENT-experiment series (Jentsch 2010).

The mean annual temperature at the site is 8.2°C with a mean annual precipitation of 724 mm, which is distributed bimodally with two major peaks in June/July and December/January (data : German Weather Service).

Subspecies and Provenances

Seeds of six provenances of *Pinus nigra* were obtained and brought to germination at the *Bavarian Institute for Forest Seeding and Planting (ASP)* in Teisendorf, Germany in April 2008. The provenances stem from autochthonal populations, except the provenance from Northern Italy for which the status is unclear and the German provenance, which most probably has an Austrian origin. Two provenances (Italy-S, France) belong to the subspecies *laricio*, the others (Germany, Austria, Italy-N, Serbia) are attributed to the subspecies *nigra*.

Table. 1: Site information (DE Germany, AT Austria, SRB Seerbia, IT Italy, FR France) for the provenances of Pinus nigra used in the EVENT 3-Experiment (Huber, 2011). Temperature (T), precipitation (P) and precipitation of warmest quarter (PJJA) represent long-term mean annual values (source: WorldClim).

Location		Ssp.	Autochthonous	Lat	Lon	Elevation (m.a.s.l.)	T (°C)	P (mm)	P JJA(mm)
DE	Zellingen	nigra	no	49°53'17"	09°43'16"	290	9.2	587	206
AT	Dreistetten	nigra	yes	47°46'00"	16°11'00"	369	8.4	712	256
SRB	Kremanske	nigra	yes	43°49'39"	19°35'22"	866	8.7	964	265
IT-N	Triestino (Carso)	nigra	unclear	45°42'00"	13°49'00"	372	11.4	1212	301
IT-S	Calabria (Sila Cosenza)	laricio	yes	39°18'08"	16°20'22"	1500	9	1300	105
FR	Ponteils-et-Bresis	laricio (Corse)	yes	44°24'18"	03°58'39"	581	10.8	745	171

In April 2009, the seedlings were transported to Bayreuth and individually planted into 4-litre pots in a sandy loam substrate from a local forest top-soil dug-out (pH 7.27, total C 1.89%, total N 0.15%, plant-available $NH4^+$1.79 mg L^{-1}, plant-available $NO3^-$ 22.50 mg L^{-1}).

Individuals were selected randomly for each provenance or subspecies from all living plants at planting date. Mean plant size at the start of the experiment was 12.2 cm (± 2.5 cm SD).

Experimental Design

The potted individuals were exposed to a temperature treatment (warming and control) and to a precipitation treatment (extreme summer drought and control). The two climate treatments were fully crossed, resulting in four climate manipulations (control, drought, warming, warming and drought), which were replicated three times, resulting in 12 experimental units in total. The provenance treatment was nested within each experimental unit, resulting in a split-plot design. Each provenance was further replicated with seven plants per experimental unit (nested replicates), resulting in 21 individuals per factorial combination of the three-factorial design and 672 plants overall.

Each experimental unit was covered by one large rain-out shelter constructed of a steel frame (GlasMetall Riemer GmbH, Rahden-Sielhorst, Germany) and covered with a transparent polyethylene sheet (0.2 mm, SPR5; Hermann Meyer KG, Rellingen, Germany). The edge of the rain-out shelters was 80 cm tall and permitted the penetration of nearly 90% of the photosynthetically active radiation.

The control precipitation regime simulated the local daily 30-year average precipitation. The application was done twice a week with collected rain water.

In 2009 the drought treatment consisted of a 42-day period without irrigation, which represents a local drought event with a statistical recurrence probability of 1000 years. The treatment started on May 27th 2009. Soil moisture was measured hourly over the whole duration of the experiment using three ECH20 EC-5 moisture sensors (Decagon Devices,Inc., USA) per treatment. The permanent wilting point was determined via soil type using a pedological soil survey manual (Ad-hoc-AG Boden 2005). Approximately three weeks after the start of the treatment soil moisture dropped below the permanent wilting point (pF = 4.2)

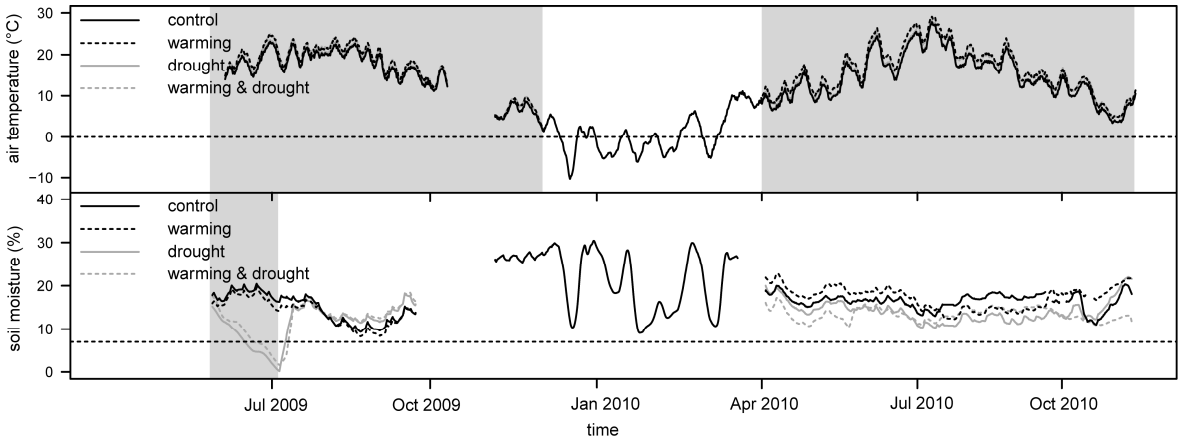

Figure 1: Air temperature at plant height and soil moisture over the two years of manipulations. From October 2009 to April 2010 no warming treatment took place. However, the plants were under snow cover, so they were sheltered from the temperature minimums in winter 09/10. In the upper graphic (air temperature) the dotted line represents 0°C, in the lower one (soil moisture) the dotted line represents approximately the permanent wilting point (pF = 4.2). The grey area represents the warming treatment in the upper graphic and the drought period in the lower.

In the re-watering phase after the drought period each individual received 240 ml and another 300 ml four days after the first re-watering in order to prevent the soil moisture from dropping below the permanent wilting point again. After this, the pots were watered according to the control precipitation treatment.

In the second year (2010), no drought treatment was applied and all plants received the same watering in order to examine the long-term effects of the previous year (2009) experimental drought event.

The warming treatment was applied from May 26th until October 25th in 2009 and from April 1st to December 7th in 2010. Warming was affected passively by wind-shelters, which reduced wind speed by 70 %, and by black floor-covers in contrast to white floor covers. This increased the average air temperature in the warming treatment at plant height by 1.5 K on average (Figure 2) compared to control. In the second year additional IR-lamps were installed (IR-radiation with approximately 30 W per m²), which only resulted in a warming by 1.6 K on average (Figure 2). Within the control treatment the temperatures were 1.5K higher on average than the ambient conditions outside of the experimental units. The moderate additional warming effect due to IR-lamps most probably resulted from different weather patterns in the second year, as the warming effect due to passive means was most pronounced under direct sun light. The fourth treatment was a combination of drought and warming. The

additional warming elevated the drought treatment effect by reducing the soil moisture by about 1.5% of total soil moisture on average compared to the drought only treatment (Figure 2).

During the winter 2009/2010, plants were kept outside the shelters in-ground in a sand bed from October to April and received natural precipitation.

Response Parameters

In 2009 the height of the plants was measured at planting time in April and after the growing period in October 2009. In 2010 height was measured again at the end of October. Increment was calculated by subtracting the initial value from the value at the end of the growing period. Individuals that died after the first year were counted with increment set to zero for the second year. In order not to underestimate height growth due to the zero increment of the dead individuals, the model was run again with the dead individuals left out completely and yielded similar results.

The number of lateral shoots was recorded in both years at the end of the vegetation period. Mortality was quantified at the end of both years by counting the living individuals.

For the phenological analyses, the date of the start (needles clearly visible in leaf sheath) and the end (needles as long as or longer than last year's needles) of foliation was recorded for each individual in 2010. Phenological data for 2009 is missing because the foliation process was already in progress when we received the plants.

Statistics

Analysis of Variance (ANOVA) combined with linear mixed effect models (package *nlme*) were applied to test for the main and interactive effects of the three factors drought, warming and provenance on height growth, number of shoots and phenology. Data was tested for homogeneity of variance and normality of the residuals prior to analysis and met the prerequisites for linear models. The experimental unit was included in the model as a random factor, accounting for the split-plot design (Pinheiro & Bates 2004). Post-hoc tests were carried out with the General Linear Hypothesis function *glht* (package *multcomp*) (Bretz 2010).

The main and interactive effects of the three factors comproising of drought, warming and provenance on mortality were tested by Analysis of Variance (ANOVA) combined with a Cox proportional hazards regression model (package *survival*) (Therneau 2000). Furthermore,

to verify the results of the Cox proportional hazards regression model, generalized linear mixed models using a binomial distribution were applied. The experimental unit was included as a random factor again. Post-hoc tests were carried out with the General Linear Hypothesis function *glht.* All statistical analyses were conducted with the software R.2.13.0 (R Development Core Team, 2011)

Results

Growth

Absolute height growth differed significantly between provenances in both years of the experiment (ANOVA: 2009: $F = 6.1$, $p = < 0.001$; 2010: $F = 3.9$, $p = 0.002$; Fig. 2 a and b). Especially the French provenance exhibited significantly less increment than the two Italian provenances in 2009 and significantly less than the Southern Italian provenance in 2010.

No significant differences between the provenances were found for relative growth (ANOVA: 2009: $F = 2.1$, $p = 0.065$; 2010: $F = 0.9$, $p = 0.277$; Fig. 2 c and d).

For both, absolute and relative growth, no significant treatment effect was detected in the first year (Fig. 2 a and c). However, in the second year a strong effect of the drought of the previous year was found (ANOVA: absolute growth: $F = 3.8$, $p = < 0.001$; relative growth: $F = 15.1$, $p = 0.005$). Plants grown under the control treatment increased in height by 6.2 cm or 51% on average, whereas the plants that were exposed to the drought in 2009 only grew 3.6 cm or 30% on average (Fig. 2 b and d).

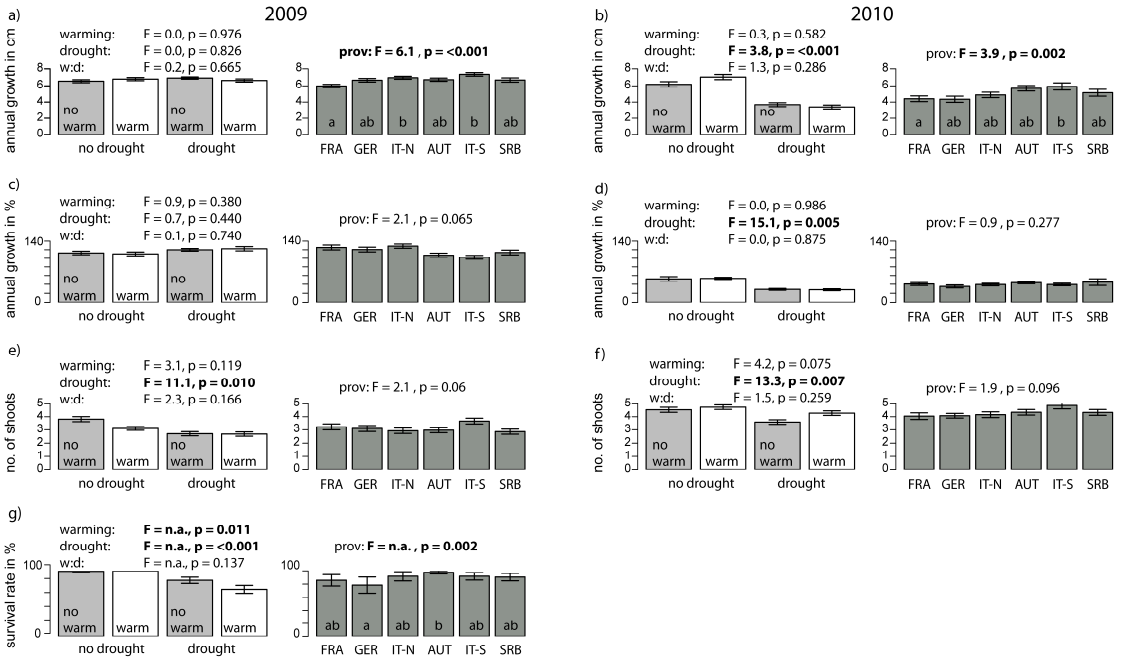

Figure 2: Response of Pinus nigra to warming, drought and provenance. a) shows the annual growth in cm as a function of treatment and provenance in 2009 and b) in 2010. c) shows the annual growth in per cent of the initial height for 2009 and d) for 2010. e) Depicts the number of shoots per individual as a response to treatment and provenance in 2009 and f) in 2010 and g) illustrates the percentage of surviving individuals in 2009. In 2010 almost all individuals survived (>99%). Lower case letters represent significant homogeneous groups as revealed by the post-hoc tests. F- and p-statistics are provided for provenance, warming, drought and the interaction between warming and drought (w:d). Significant p-values (<0.05) indicated by bold lettering. No significant interactions between provenance and weather treatments were found. The Cox proportional hazard model does not provide F-satistics. Provenances are arranged according to their geographic origin from east to west.

Warming had no significant impact on height growth, neither in 2009 (ANOVA: absolute growth: F = 0.0, p = 0.976; relative growth: F = 0.9, p = 0.380), nor in 2010 (ANOVA: absolute growth: F = 0.3, p = 0.582; relative growth: F = 0.0, p = 0.986).

Sensitivity to warming and to drought did not differ among the provenances (ANOVA: interaction between provenance and drought: 2009, absolute growth: F = 0.9, p = 0.483; relative growth: F = 2.1, p = 0.068; 2010, absolute growth: F = 1.3, p = 0.262; relative growth: F = 0.9, p = 0.478; interaction between provenance and warming: 2009, absolute growth: F = 0.8, p = 0.546; relative growth: F = 1.1, p = 0.379; 2010, absolute growth: F = 0.7, p = 0.613; relative growth: F = 0.5, p = 0.769).

Number of Shoots

The provenances did not differ significantly in terms of the number of shoots in any year (ANOVA: 2009: F = 2.1, p = 0.060; 2010: F = 1.9, p = 0.096). Drought, however, reduced the

number of shoots (ANOVA: 2009: F = 11.1, p = 0.01; 2010: F = 13.3, p = 0.007; Fig. 2 e and f). In 2009 the control plants had 3.3 shoots on average and the plants undergoing drought treatment 2.8 shoots on average. In 2010 it was 4.5 (control) to 4.1 (drought) on average.
Number of shoots was not significantly affected by warming in any year (ANOVA: 2009: F = 3.1, p = 0.119; 2010: F = 4.2, p = 0.075). Overall, the provenances showed no significant variation in their response to the weather manipulations (ANOVA: 2009: interaction between provenance and drought: F = 1.0, p = 0.413; interaction between provenance and warming F = 0.8, p = 0.554, 2010: interaction between provenance and drought: F = 0.5, p = 0.765; interaction between provenance and warming F = 0.6, p = 0.730).

Needle Phenology

The onset of needle foliation in 2010 did not differ significantly among the provenances (Fig. 3) (ANOVA: F = 1.1, p = 0.377).
The warming treatment led to a earlier bud burst. Needle foliation started 10.6 days earlier when the plants were exposed to warming compared with control individuals (ANOVA: F = 49.1, p = <0.001). Needle foliation was completed 2.3 days earlier on average for plants under warming treatment, though this was an insignificant trend at the 95% confidence level (ANOVA: F = 3.6, p = 0.079). The influence of the drought treatment on the phenological development of leaves was not significant at all (ANOVA: F = 0.2, p = 0.655).

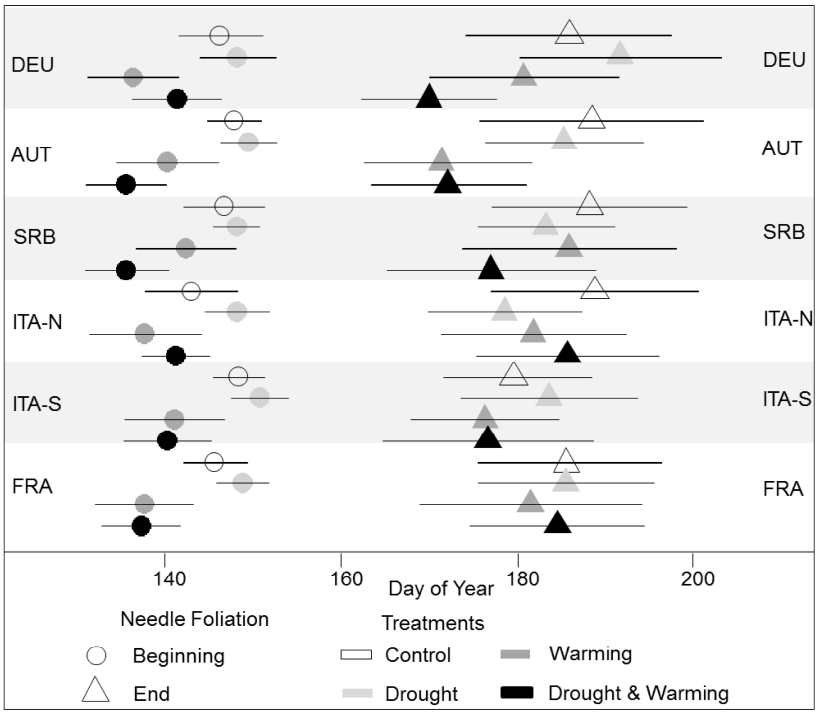

Figure 3: Needle foliation in 2010 for the different provenances. Circles represent bud burst and triangle the completion of needle flushing. The different shades of grey illustrate the four treatments. The horizontal bars represent the standard error. Warming siginificantly impacted bud burst (ANOVA: $F = 49.1$, $p = < 0.001$).

Generally, the provenances showed no significant variation in their response to the weather manipulations in terms of needle phenology (ANOVA: interaction between provenance and warming: $F = 1.3$, $p = 0.262$; interaction between provenance and drought: $F = 0.6$, $p = 0.738$).

Mortality

Provenances exhibited a significant difference in terms of mortality in 2009 (ANOVA: $p = < 0.001$. The German provenance showed the lowest survival rate (78.6%), while the Austrian provenance showed the highest survival rate (97.6%) (Fig. 2 g).

Drought significantly increased mortality (ANOVA: $p = < 0.001$). Only 79% of the plants subjected to the drought treatment survived, whereas 99.6% of the plants that were not exposed to drought (control and warming) were alive after the first season (Fig. 2 g). Warming significantly decreased surivival (ANOVA: $p = 0.011$). However, the decreased survival only occurred in combination with the drought treatment (- 13%), though no statistically significant interaction between drought and warming could be detected with any of the two statistical methods applied (ANOVA: interaction warming and drought: $p = 0.137$; Fig. 5 a, Tab. 2).

The provenances showed no significant variation in their response to the weather manipulations (ANOVA: interaction between provenance and warming: $p = 0.444$; interaction between provenance and drought: $p = 0.840$).

In 2010 mortality was negligible (<1%) and no trends based on treatment or provenance effects became visible. The GLM model qualitatively yielded the same results.

Discussion

Within-species variation in the absence of climate perturbations

Pinus nigra has a very fragmented distribution range across the (sub-) Mediterranean region. It is split into six subspecies, which are again subdivided into varieties (Isajev 2004). Especially in the western part of its range, *P. nigra* populations most likely survived the last glacial maximum in refugia (Afzal-Rafii et al. 2007), which implies a long-term separation of

populations. *P. nigra* shows a high genetic distance between populations (Scaltsoyiannes et al. 2009) and thus it can be expected that phenotypic differences also occur between populations and provenances. The different performance of the six provenances in terms of height growth in our experiment is therefore not surprising. The southern Italian provenance showed the highest total height increment in both years, whereas the performance of the French provenance was lowest in 2009 and 2010. The significant disparity between the two provenances is unexpected, because they both belong to the same subspecies *laricio* (Huber 2011). This variation in growth may indicate a distinct inter-population genetic variation even within a subspecies.

In 2009, the Northern Italian provenance performed at the same level as the Southern Italian provenance with high growth rates across all treatments, despite the fact that it originates from a site with high annual precipitation and also high precipitation during the vegetation period (Tab. 1), where a potential local adaptation should lead to a higher drought-susceptibility than for sites with low precipitation. However, Soto et al. (2010) found a positive correlation between summer precipitation and within-population diversity for *Pinus nigra*, as regions with regular precipitation would support larger and demographically more stable populations, than regions with a higher summer drought frequency. This probable high genetic diversity may be the reason for the relatively stable growth and relatively high survival rate within this provenance.

Another surprising pattern is revealed by the survival rates. The German provenance exhibits a significantly lower survival rate than the Austrian provenance. However, the German provenance was founded in 1909/1910 with seeds from Austria (Huber 2011). Climatic conditions and elevation are also similar between the places of origin of these two provenances. Nevertheless they show distinct performances, which may point again to the fact that populations strongly vary genetically, despite a close spatial relationship.

Budburst was not influenced by provenance. Though the Northern Italian provenance completed needle foliation significantly later than the other provenances, it seems that provenance or genetic differences do not influence phenological behavior as much as the temperature signal does. This is in accordance with findings for other tree species (Vitasse et al. 2009) and indicates a strong potential for adaptation to changing climatic conditions.

Species Response to Drought and warming

A reduction in summer precipitation by about 20% is projected for Central Europe according to the A1B scenario (BfG 2009), with an increased risk of drought periods (Rowell 2009).

These altered climatic conditions will have a strong impact on temperate forest ecosystems. Especially indigenous conifers will suffer most under dry summers (Kölling 2009, Lindner et al. 2010). *Pinus nigra* has to be able to cope with the projected climatic conditions, in order to come into consideration as a potential substitute for the endangered locals.

Drought was the main source of variation in growth and survival across all provenances in our experiment. Yet, considering the extremeness of the drought treatment (42 days without precipitation and more than two weeks soil moisture below the permanent wilting point), survival and growth rates remained remarkably high. Surprisingly, there was no drought effect on height growth in the first year, when the actual drought treatment took place. However, in the second year height increment strongly decreased for individuals exposed to drought in the previous year, despite no further drought treatment. Across the provenances the saplings of *P. nigra* exhibited a lagged response to water shortage by maintaining high growth rates in the drought year and a height growth drop in the subsequent year. Similar lagged effects of drought are reported by other studies. Lebourgeois (2000) found significant correlations between early radial growth and temperature (negative) and precipitation (positive) in October of the previous year in a study on climatic impacts on the growth of Corsican Pine (*Pinus nigr*a ssp. *laricio* var. *Corsicana*). Analogous results were reported by Martin-Benito et al. (2008). They show that the early wood growth of *Pinus nigra* in Southeastern Spain was negatively impacted by a previous year drought event. During water stress, newly fixed carbon is rather used for osmotic adjustment to improve drought resistance or is allocated to root growth in order to acquire more water. This physiological response may reduce carbohydrate reserves which are necessary for next year's growth (Lebourgeois 2000, Martin-Benito et al. 2008). Such lagged responses have been confirmed for other conifers (Andreu et al. 2007, de Grandpre et al. 2011). However, all these focused on adult trees, with significant storage capacity for carbohydrates. A delayed growth response for seedlings, as presented in our study is a novel finding. Compared to adult trees, seedlings have less storage capacities, due to a smaller share of woody tissue. Therefore, a reduced carbohydrate production or a reallocation of assimilates should lead to a more immediate height growth reaction. Maintaining a high growth rate despite water stress might therefore be related to the strong increase in mortality of juvenile stages during and after water stress. Furthermore the drought period might alter soil organic matter decomposition and nitrogen mineralization processes, what can lead to carry-over effects into the next years (van der Molen et al. 2011).

The survival of the pine seedlings in 2009 depended significantly on water conditions. Nevertheless the overall survival rate was relatively high (79%) in the drought treatments,

considering the young age of the seedlings (one year). The warming treatment amplified the negative drought effect on survival, confirming the assumption that in regions with low precipitation and water shortage during the vegetation period additional warming makes water stress more severe (Martin-Benito et al. 2010, McLane et al. 2011), probably due to increased evapotranspiration.

In general, the two reactions to drought, die-off and growth reduction, happened temporally separated. Drought-induced mortality solely took place in the drought year, whereas reduction in growth did not become significant until before the following year. It seems that most of the plants could deplete stored carbohydrates to secure survival during the first year, but these plants lacked in reserves necessary for growth in the next year. However, to explain the physiological reasons for this behavior, it is important for upcoming studies to provide a higher temporal solution of growth and to determine the exact cause of death (cavitation or starvation (Adams et al. 2009)).

The number of lateral shoots was also significantly negatively impacted by the drought treatment. However, effects of the treatment became already visible in the first year and maintained until the year after the treatment. As shoot growth takes place later in the growing season, the influence of current year conditions is higher (Lebourgeois 2000, Martin-Benito et al. 2008) than for height growth.

The warming treatment led to an earlier bud burst across all provenances. This might lead to a higher annual increment, due to a prolonged period with more photosynthetically active biomass. Yet, such a response to increased temperatures could not be observed in our experiment. The caveat of earlier budburst is the increased late frost sensitivity, especially as the period until completion of foliation was considerably elongated by the warming treatment. Viveros-Viveros et al. (2009), Kandemir et al. (2010) or Kreyling et al. (2011 a) assume that the impacts of late frost will grow in a warmer world as it may hit vegetation that is further developed. They point out that the timing of such events is crucial, as foliage is especially susceptible shortly after bud burst. Moreover, a prolonged needle maturation period might make the tree more susceptible to herbivory, as younger needles are usually more nutritious and contain less defensive compounds and therefore make up a superior diet for insects (Ruusila 2005). Even if we could not find any differences in timing of flushing between the provenances, these aspects have to be taken into deeper consideration before introducing this species into novel climatic or more late frost prone regions in Central Europe.

In general the additional warming treatment had surprisingly little influence on performance, indicating that water shortage is the main driver of declining performance. The negligible

effect of warming could be due to the fact that we only applied a moderate increase in temperature (about 1.6 K compared to Control). The warming was not intended to be extreme, but to simulate the expected average background temperature in the second half of the 21st century. Extreme heat waves are expected to occur with probably similar effects on vegetation than drought events (Clark et al. 2010, DeBoeck 2010).

Provenance-specific Response to Drought and Warming

The provenances responded uniformly to our drought and warming treatments, implying that no variation in sensitivity to climate change occurs among provenances. Consequently, drought or mean temperature does not appear to impose significant selective pressure in *Pinus nigra* populations up to now. High drought tolerance across populations (Lebourgeois et al. 1998, Martinez-Vilalta et al. 2002) seems to prevent strong selection. Only the German provenance exhibited a non significant trend towards higher drought induced mortality. Yet, a high mortality rate can support a faster adaptation of forests to changing climatic conditions, than earlier maturation age or dispersal ability, as the most drought-tolerant individuals survive (Kuparinen et al. 2010). The relatively good performance of the surviving individuals of the German provenance may underline these findings.

Our findings are supported by previous studies for other pine species. Lamy et al. (2011) found no significant differences in cavitation resistance of six populations of *Pinus pinaster* in a provenance trial in Spain. Similiar results were yielded by Maherali et al. (2000) for *Pinus ponderos,* which showed no signs of intraspecific variation in terms of cavitation vulnerability as well. In a common garden experiment with two irrigation regimes including two montane and two desert populations of *Pinus ponderosa* between-population differences in biomass-allocation and hydraulic architecture did not correlate with the climate at the places of origin of the populations (Maherali et al. 2002). Martinez-Vilalta et al. (2009) detected local adaptation to drought in 12 *Pinus sylvestris* populations for some hydraulic traits, but for cavitation vulnerability and leaf physiology no significant interaction with climate dryness could be accounted for."

However, local adaptations to climatic conditions could already be detected for *Pinus nigra* in terms of frost-tolerance, showing a correlation with minimum temperatures at the site of origin (Kreyling et al. 2012). Winter conditions might therefore impose a higher selective pressure on *Pinus nigra* populations than the climate during the vegetation period, and winter climate change might consequently play a much more important role in population dynamics than previously assumed (Kreyling 2010).

The transplantation or assisted colonization of pre-adapted provenances of key tree species within their natural distribution range might be an adequate tool for the conservation of ecosystem functioning and services in the face of climate change (Kreyling et al. 2011 b). However, especially Central European conifer species, like *Picea abies* will most probably face climatic conditions that they have not yet experienced within their distribution range (Kölling 2007; Kölling 2009) and pre-adapted ecotypes might therefore not exist. *Pinus nigra*, as a drought-tolerant species, might be better adapted to the future conditions, and might thus serve as a substitute for conifers at dry sites in Central Europe (Huber 2011). The introduction or transplantation of exotic species has a long tradition in forestry. Still, numerous examples of failed transplantation warn against rushed action (Zobel et al. 1987).

Our results suggest that looking for a single provenance, which is "best-suited" to the future climate and other site conditions at a certain location, might be too narrowly considered. We did not find any local adaptations in growth and survival response to drought and warming between provenances on a continental scale. Yet, we only included a relatively small selection of provenances in our experiment and did not test them for the multitude of possible changing climatic variables, such as heavy rain, storms or frost events. For example the Italian provenances, which performed well in terms of growth and survival in this study, exhibit a low cold hardiness (Kreyling et al. 2012), which might make them susceptible to frost damage. This means a provenance that copes well with one climate extreme might react negatively to another one. Furthermore we did not include the important aspect of forest pests. Herbivory and diseases might become increasingly important aspects of forest ecology in the face of climate change (Watt et al. 2011) and need to be taken into consideration before the introduction of novel species. Also, economic aspects of tree growth like wood quality and straightness in growth need to be examined in standardized provenance trials (Huber 2011), which help to detect the whole spectrum of species-specific responses (Kreyling et al. 2011 a). Still, results of such experiments might noticeably change when interacting drivers like multiple climatic variables or biotic interactions are considered in a multi-factor experiment.

Conclusions and Outlook

Delayed growth response after drought in juvenile forest trees needs to be considered in field observations and common garden experiments when studying the consequences of climatic change. Short-term studies have to be conducted with great care in order to avoid misinterpretation of initial drought tolerance.

Pinus nigra shows a high drought tolerance and appears well adapted to the future climate conditions in Central Europe. Provenances of *P. nigra* differ strongly in their performance, while exhibiting a surprisingly uniform sensitivity to drought and mean temperature showing no sign of local adaptation. These results suggest that the search for a "best-adapted" provenance for transplantation based on equivalent climates is not useful, especially regarding the multitude of possible changing climatic parameters and their interactions. Furthermore, herbivory and diseases might become increasingly important in the face of climate change. We therefore suggest management actions aiming at the conservation or increase of genetic diversity of populations, e.g. by promotion of natural regeneration in native stands or intermixing of different genotypes, as this enables adaptation to novel growing conditions.

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References

Adams, H.D., Guardiola-Claramonte, M., Barron-Gafford, G.A., Villegas, J.C., Breshears, D.D., Zou, C.B. et al., 2009. Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. Proc. Natl. Acad. Sci. USA 106, 7063-7066.

Afzal-Rafii, Z., Dodd, R.S., 2007. Chloroplast DNA supports a hypothesis of glacial refugia over postglacial recolonization in disjunct populations of black pine (*Pinus nigra*) in western Europe. Mol. Ecol. 16, 723-736.

Aguinagalde, I., Llorente, F., Benito, C., 1997. Relationships among five populations of European black pine (Pinus nigra ARN.) using morphometric and isozyme markers. Silvae Genet. 46, 1-5.

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M. et al., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For. Ecol. Manag. 259, 660-684.

Andreu, L., Gutiérrez, E., Macias, M., Ribas, M., Bosch, O., Camarero, J.J., 2007. Climate increases regional tree-growth variability in Iberian pine forests. Global Change Biol. 13, 804-15.

BfG, 2009. REMO-BfG. Max Planck Institue for Meteorology on behalf of Bundesanstalt für Gewässerkunde (BfG), Am Mainzer Tor 1, 56068, Koblenz, Germany.

Biel, C., Save, R., Habrouk, A., Espelta, J.M., Retana, J., 2004. Effects of restricted watering and CO_2 enrichment in the morphology and performance after transplanting of nursery-grown *Pinus nigra* seedlings. Hort Science 39, 535-540.

Bolte, A., Degen, B., 2010. Forest adaptation to climate change - options and limitations. Landbauforsch. 60, 111-117.

Bretz F., Hothorn T.,Westfall P., 2010. Multiple Comparisons Using R, CRC Press, Boca Raton.

Ad-hoc-AG Boden der Staatlichen Geologischen Dienste und der Bundesanstalt für Geowissenschaften, 2005. Bodenkundliche Kartieranleitung, Hannover

Clark, R.T., Murphy, J.M., Brown, S.J., 2010. Do global warming targets limit heatwave risk? Geophys. Res. Lett. 37.

De Boeck, H.J., Dreesen, F.E., Janssens, I.A., Nijs, I., 2010**.** Climatic characteristics of heat waves and their simulation in plant experiments. Global Change Biol. 16, 1992-2000.

De Grandpre, L., Tardif, J.C., Hessl, A., Pederson, N., Conciatori, F., Green, T.R. et al., 2011. Seasonal shift in the climate responses of *Pinus sibirica, Pinus sylvestris*, *and Larix sibirica* trees from semi-arid, north-central Mongolia. Can. J. Forest Res. 41, 1242-1255.

Hosius, B., Leinemann, L., Konnert, M., Bergmann, F., 2006. Genetic aspects of forestry in the central Europe. Eur. J. Forest Res. 125, 407-417.

Huber, G., 2011. Neue Tests für Schwarzkiefern-Herkünfte in Bayern im Hinblick auf den Klimawandel. Internationaler Bayerischer Schwarzkiefern-Herkunftsversuch 2009/2010, Teisendorf.

Isajev, V., Fady, B., Semerci, H., Andonovski, V., 2004. EUFORGEN Technical guidelines for the genetic conservation and use of European Black pine (*Pinus nigra*), Rome, Italy.

Jagielska, A., Cwalina, M., Prus-Glowacki, W., 2007. Genetic diversity of the black pine‘s stands (*Pinus nigra* Arn.). Sylwan 151, 23-31.

Jentsch, A., Beierkuhnlein C., 2008 Research frontiers in climate change: effects of extreme meteorological events on ecosystems. CR. Geoscience 340, 621-628.

Jentsch, A., Beierkuhnlein C., 2010 Simulating the future - responses of ecosystems, key species, and european provenances to expected climatic trends and events. Nova Acta Leopoldina NF 112, 384, 89-98.

Jentsch, A., Kreyling, J., Beierkuhnlein, C., 2007. A new generation of climate-change experiments: events, not trends. Front. Ecol. Environ. 5, 365-374.

Kandemir, G.E., Kaya, Z., Temel, F., Onde, S., 2010. Genetic Variation in Cold Hardiness and Phenology Between and Within Turkish red pine (*Pinus brutia Ten.*) populations: implications for seed transfer. Silvae Genet. 59, 49-57.

Kölling, C., 2007. Klimahüllen für 27 Waldbaumarten. AFZ Der Wald, 1242-1245.

Kölling, C., 2009. Überlegungen zum Risiko des Fichtenanbaus in Deutschland vor dem Hintergrund des Klimawandels. Forstarchiv 80, 42-54.

Kreyling, J., 2010. Winter climate change: a critical factor for temperate vegetation performance. Ecology 91, 1939-1948.

Kreyling, J., Thiel, D., Nagy, L., Jentsch, A., Huber, G., Konnert, M. et al., 2011a. Late frost sensitivity of juvenile *Fagus sylvatica* L. differs between southern Germany and Bulgaria and depends on preceding air temperature. Eur. J. Forest Res. 19. DOI: 10.1007/s10342-011-0544-y

Kreyling, J., Bittner, T., Jaeschke, A., Jentsch, A., Steinbauer, M.J., Thiel, D. et al., 2011b. Assisted colonization: a question of focal units and recipient localities. Restor. Ecol. 19, 433-440.

Kreyling J., Wiesenberg G., Daniel, T., Wohlfahrt C., Huber, G., Walter, J. et al., 2012. Cold hardiness of *Pinus nigra Arnold* as influenced by geographic origin, extreme summer drought and gradual warming. Environ. Exp. Bot., 78, 99-108.

Kuparinen, A., Savolainen, O., Schurr, F.M., 2010. Increased mortality can promote evolutionary adaptation of forest trees to climate change. For. Ecol. Manage. 259, 1003-1008.

Lamy, J.-B., Bouffier, L., Burlett, R., Plomion, C., Cochard, H., Delzon, S., 2011. Uniform selection as a primary force reducing population genetic differentiation of cavitation resistance across a species range. PLoS One 6, e23476.

Lebourgeois, F., Levy, G., Aussenac, G., Clerc, B., Willm, F., 1998. Influence of soil drying on leaf water potential, photosynthesis, stomatal conductance and growth in two black pine varieties. Ann. Forest Sci. 55, 287-299.

Lebourgeois, F., 2000. Climatic signals in earlywood, latewood and total ring width of Corsican pine from western France. Ann. Forest Sci. 57, 155-164.

Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, A., Garcia-Gonzalo, J. et al., 2010. Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For. Ecol. Manage. 259, 698-709.

Lu, P., Man, R., 2011. Assessment of assisted migration effects on spring bud flush in white spruce (*Picea glauca [Moench] Voss*) seedlings. Forest. Chron. 87, 391-397.

Lucic, A., Mladenovic-Drinic, S., Stavretovic, N., Isajev, V., Lavadinovic, V., Rakonjac, L. et al., 2010. Genetic diversity of Austrian Pine (*Pinus Nigra ARNOLD*) populations in Serbia revealed by RAPD. Arch. of Biol. Sci. 62, 329-336.

Maherali, H., Delucia, E.H., 2000. Xylem conductivity and vulnerability to cavitation of ponderosa pine growing in contrasting climates. Tree Phys. 20, 859-867.

Maherali, H., Williams, B.L., Paige, K.N., Delucia, E.H., 2002. Hydraulic differentiation of Ponderosa pine populations along a climate gradient is not associated with ecotypic divergence. Func. Ecol. 16, 510-521.

Maracchi, G., Sirotenko, O., Bindi, M., 2005. Impacts of present and future climate variability on agriculture and forestry in the temperate regions: Europe. Clim. Change 70, 117-135.

Martin-Benito, D., Cherubini, P., del Rio, M., Canellas, I., 2008. Growth response to climate and drought in *Pinus nigra Arn*. trees of different crown classes. Trees-Struct. Funct. 22, 363-373.

Martin-Benito, D., del Rio, M., Canellas, I., 2010. Black pine (*Pinus nigra Arn.*) growth divergence along a latitudinal gradient in Western Mediterranean mountains. Ann. Forest Sci. 67, 401-401.

Martinez-Vilalta, J., Pinol, J., 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. For. Ecol. Manag. 161, 247-256.

Martinez-Vilalta, J., Cochard, H., Mencuccini, M., Sterck, F., Herrero, A., Korhonen, J.F.J. et al., 2009. Hydraulic adjustment of Scots pine across Europe. New Phytol. 184, 353-364.

Mataruga, M., Haase, D.L., Isajev, V., 2010. Dynamics of seed imbibition and germination of Austrian pine *(Pinus nigra Arnold*) from extreme habitat conditions within five Balkan provenances. New For. 40, 229-242.

McKenney, D., Pedlar, J., O'Neill, G., 2009. Climate change and forest seed zones: Past trends, future prospects and challenges to ponder. For. Chron. 85, 258-266.

McLane, S.C., Daniels, L.D., Aitken, S.N., 2011. Climate impacts on lodgepole pine (*Pinus contorta*) radial growth in a provenance experiment. For. Ecol. Manag. 262, 115-123.

Menzel, A., Fabian, P., 1999. Growing season extended in Europe. Nature 397, 659.

Min, S.-K., Zhang, X., Zwiers, F.W., Hegerl, G.C., 2011. Human contribution to more-intense precipitation extremes. Nature 470, 378-381.

Nathan, R., Horvitz, N., He, Y., Kuparinen, A., Schurr, F.M. & Katul, G.G., 2011. Spread of North American wind-dispersed trees in future environments. Ecol. Lett., 14, 211-219.

Pinheiro, J.C., Bates, D.M., 2004. Mixed-effects models in S and S-PLUS, Springer, New York.

R Development Core Team, 2011. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria.

Rowell, D.P., 2009. Projected midlatitude continental summer drying: North America versus Europe. J. Climate 22, 2813-2833.

Ruusila, V., Morin, J. P., van Ooik, T., Saloniemi, I., Ossipov, V., Haukioja, E., 2005. A short-lived herbivore on a long-lived host: tree resistance to herbivory depends on leaf age. Oikos 108, 99-104.

Scaltsoyiannes, A., Tsaktsira, M., Pasagiannis, G., Tsoulpha, P., Zhelev, P., Iliev, I. et al., 2009. Allozyme variation of European Black (*Pinus nigra Arnold*) and Scots pine (*Pinus sylvestris L.*) populations and implications on their evolution: A comparative study. J. Biol. Res.-Thessalon. 11, 95-106.

Schaberg, P.G., DeHayes, D.H., Hawley, G.J., Nijensohn, S.E., 2008. Anthropogenic alterations of genetic diversity within tree populations: Implications for forest ecosystem resilience. For. Ecol. Manag. 256, 855-862.

Seho, M., Kohnle, U., Albrecht, A., Lenk, E., 2010. Growth analyses of four provenances of European Black Pine (*Pinus nigra*) growing on dry sites in southwest Germany (Baden-Wuerttemberg). Allg. Forst Jagsdztg. 181, 104-116.

Soto, A., Robledo-Arnuncio, J.J., Gonzalez-Martinez, S.C., Smouse, P.E., Alia, R., 2010. Climatic niche and neutral genetic diversity of the six Iberian pine species: a retrospective and prospective view. Mol. Ecol. 19, 1396-1409.

Therneau, T., Grambsch, P., 2000. Modeling survival data: extending the cox model. Springer, New York.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C. et al., 2004. Extinction risk from climate change. Nature 427, 145-148.

van der Molen, M. K., Dolman, A. J., Ciais, P., Eglin, T., Gobron, N., Law, B. E., Meir, P., Peters, W., Phillips, O. L., Reichstein, M., Chen, T., Dekker, S. C., Doubkov´a, M., Friedl, M. A., Jung, M., van den Hurk, B. J. J. M., de Jeu, R. A. M., Kruijt, B., Ohta, T., Rebel, K. T., Plummer, S., Seneviratne, S. I., Sitch, S., Teuling, A. J., van der Werf, G. R., and Wang, G., 2011. Drought and ecosystem carbon cycling, Agric. For. Meteorol. 151, 765-773.

Varelides, C., Brofas, G., Varelides, Y., 2001. Provenance variation in *Pinus nigra* at three sites in Northern Greece. Ann. For. Sci. 58, 893-900.

Vitasse, Y., Delzon, S., Dufrene, E., Pontailler, J.-Y., Louvet, J.-M., Kremer, A. et al., 2009. Leaf phenology sensitivity to temperature in European trees: Do within-species populations exhibit similar responses? Agric. For. Meteorol. 149, 735-744.

Viveros-Viveros, H., Saenz-Romero, C., Jesus Vargas-Hernandez, J., Lopez-Upton, J., Ramirez-Valverde, G., Santacruz-Varela, A., 2009. Altitudinal genetic variation in *Pinus hartwegii Lindl*. I: Height growth, shoot phenology, and frost damage in seedlings. For. Ecol. Manag. 257, 836-842.

Watt, M.S., Ganley, R.J., Kriticos, D.J., Manning, L.K., 2011. Dothistroma needle blight and pitch canker: the current and future potential distribution of two important diseases of Pinus species. Can. J. For.Res. 41, 412-424.

Zobel, B.J., van Wyk, G., Stahl, P., 1987. Growing exotic forests, John Wiley & Sons, New York.

6.3. Manuscript 3:

Different reactions of central and marginal provenances of Fagus sylvatica to experimental drought

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Keywords

Extreme events, local adaptation, phenotypic plasticity, plant-climate interactions, provenance trial, within-species diversity

Abstract

Climate extremes are expected to increase in frequency and magnitude as a consequence of global warming, threatening the functioning, services and goods of forest ecosystems. Across Europe, the ecologically and economically important tree species Fagus sylvatica is expected to suffer particularly under such conditions.

The regional introduction of provenances from drier and warmer climates is one option to adapt beech forest ecosystems to these adverse effects of climate change. Marginal populations from the drought-prone southern and north-eastern edges of the species'

distribution come into focus in search of suitable candidates for Central European deciduous forests.

Here, we test three marginal provenances (Spain, Bulgaria and Poland) and three provenances from the centre of the distribution range (Germany) for their response to drought in two different soil types (sand, loam) in a full-factorial common-garden experiment in Landau, Germany.

Drought impacted all growth parameters negatively (leaf damage +22% (percentage points), height -40% and diameter increment -41%) and the sandy substrate exacerbated this effect. However, provenances differed in their response to drought and soil type. Evidence for a local adaptation to summer drought was detected, especially in terms of mortality rates. The Bulgarian and Spanish provenance showed a stable performance under drought conditions (BG -27% in diameter increment; ES -32%), compared to the Polish (-48%) or the most sensitive German provenances (-57%), yet for Bulgaria on a low level of total increment. This may indicate a trade-off between drought-tolerance and growth.

Therefore, a sole focus on drought-resistant marginal provenances seems to not be conducive, as they might be less adapted to other climatic factors, e.g. frost, as well. However, intermixed with local Central European provenances these may act as functional insurance in future drought-prone forest stands.

Introduction

Alongside an increase in average temperature, Global Warming is expected to trigger an increase in magnitude and frequency of climatic extremes such as prolonged drought events (IPCC 2012). This will especially affect the functioning of ecosystems with long-living organisms, such as forests, and may lead to a declining provision of forest goods and services (Allen et al. 2010, Lindner et al. 2010). The velocity of these changes in ecosystem functioning may exceed the natural dispersal rates of trees and the speed of evolutionary adaptation processes (Bertrand et al. 2011, Chen et al. 2011).

Fagus sylvatica is a naturally dominant and economically important, but drought-susceptible tree species in Central Europe that deserves special attention in the face of these changing conditions (Fotelli et al. 2009). Even though F. sylvatica is considered a high-risk species in the face of climate change (Ohlemuller et al. 2006, Scherrer et al. 2011), the area stocked with F. sylvatica constantly increases in Central Europe/Germany due to forest conversion from coniferous to mixed stands. According to Polley et al. (2009) beech is the species in Germany

with the highest increase in stocked area between 2002 and 2007 (83.000ha). Drought events most probably determine the southern edge of the distribution range (Jump et al. 2006). Especially at lower elevations growth and distribution of F. sylvatica is expected to decline at its southern limits (Matyas et al. 2009, Hlasny et al. 2011,Jezik et al. 2011) and on xeric sites in Central Europe (Czucz et al. 2011). The observed recent decline in beech forest productivity in France could be linked to low water availability, especially in early summer (Lebourgeois et al. 2005, Charru et al. 2010). Furthermore Piovesan et al. (2008) showed that basal area increment of European beech stands decreased with decreasing water availability in the Apennine Mountains in Italy. The exceptional 2003 drought period over Central Europe generated distinct negative effects on the performance of F. sylvatica (Czajkowski et al. 2005, Leuzinger et al. 2005, Betsch et al. 2011). Moreover it seems that F. sylvatica loses its competitive advantage to less drought-sensitive species, e.g. Quercus petraea, under water limited conditions in Southern and South-Eastern part of its distribution range (Bonn 2000, Fotelli et al. 2001, Friedrichs et al. 2009, Clark et al. 2011, Scharnweber et al. 2011), whereas positive impacts of climate change on competition may prevail on the northern distribution edge in Sweden (Bolte et al. 2010). Drought periods also increase pathogen- and fungi-susceptibility of F. sylvatica, as shown by Jung (2009) for the post-2003 years in a Bavarian beech stand. Therefore, Rennenberg et al. (2004) and Gessler et al. (2007) consider the focus on F. sylvatica in the ongoing forest conversion as very risky.

However, F.sylvatica, with its wide geographic distribution, covers a broad range of climatic conditions (see Kölling 2007). The introduction of F. sylvatica provenances from warmer, more drought-prone regions, especially from the edges of its distribution range to Central Europe might therefore be one potential adaptation tool to dampen the adverse effects of future climatic environments (Bolte et al. 2009, Kreyling et al. 2011). The genetic composition of forest trees determines the phenotypic plasticity and with this their adaptive capacity to environmental stressors such as drought (Schaberg et al. 2008, Matyas et al. 2009). In Central Europe, F. sylvatica exhibits a high genetic diversity within populations (Konnert 1995, Vornam et al. 2004), whereas on a continental scale the genetic differentiation between populations becomes more distinct (Comps et al. 1990, Magri et al. 2006). These differences in genetic configuration between populations, most likely, display differences in adaptive capacity between provenances. In numerous provenance-trials over the last decade, a contrasting performance of provenances from different geographic origins has been demonstrated (e.g. von Wuehlisch et al. 1995). Furthermore, it was shown that different provenances exhibit specific responses to abiotic stressors such as late frosts (Kreyling et al.

2012) or drought (Schraml und Rennenberg 2000, Peuke et al. 2006). In a European –wide provenance-trial network, macroclimatic adaptation could be detected: The performance of different provenances was negatively correlated with climatic distance (absolute differences in certain climatic parameters) between test-site and origin of the provenance (Matyas et al. 2009). Czajkowski and Bolte (2006) found a significantly higher transpiration and therefore higher water stress under drought conditions for German and West-Polish provenances than for provenances from the more continental Central-Polish eastern distribution edge of F. sylvatica. In the extraordinary dry year 2003, NW-Greek beech populations only experienced mild drought stress compared to the reported damages in beech forests in Central Europe, despite comparable climatic conditions (Fotelli et al. 2009), thereby indicating a local adaptation of Greek populations to water shortages. Therefore, such marginal populations from the dry edges of the current distribution range of F. sylvatica may come into focus in search of drought-resistant ecotypes (Rose et al. 2009, Kreyling et al. 2011) The susceptibility to climatic stressors such as drought, however, strongly depends on the interaction with additional biotic or abiotic factors (Messaoud and Chen 2011). The impact of drought and water stress on the performance of F. sylvatica depends on air ozone concentration (Pollastrini et al. 2010), carbon dioxide concentrations (Penuelas et al. 2008), light availability (Czajkowski et al. 2005, Lof et al. 2005) and soil chemistry (Weitner et al. 2007). Evidence suggests that, in general, drought reduces nutrient availability and uptake in forest soils (Rennenberg et al. 2009, Kreuzwieser and Gessler 2010). The interactive effects of soil nutrient balance and water availability on fine roots, microorganisms and soil nutrient cycling processes are poorly understood (Kreuzwieser and Gessler 2010). However, especially for F. sylvatica which grows on various soil types throughout its distribution range (Ellenberg and Leuschner 2010), the occurrence of drought conditions or water stress is a complex interactive mechanism between climatic and edaphic factors (Gaertner et al. 2008).

Considering the reported negative impacts of drought on the performance of F. sylvatica and the intraspecific variability in drought response, it is important to know whether certain provenances of F. sylvatica are less susceptible to these negative effects and whether site factors such as soil type influence specific responses. This knowledge is crucial to assess the potential of selective transplanting of drought-resistant provenances as a tool to dampen negative impacts of such climate extremes in the course of climate change.

Here, seedlings of six provenances of F. sylvatica, three from the centre of its distribution range end three from the margins (SE, SW, NE) were exposed to drought in two different soil

types (with contrasting nutrient availability and field capacity) in a full factorial common garden experiment. We hypothesized, that (1) provenances differ in the extent of leaf damage, height and diameter growth and (2a) drought and (2b) sandy soil substrate negatively influence these performance indicators. We further assumed that (3) marginal populations are less drought sensitive, and we expected (4) that soil conditions and drought interactions significantly affect plant performance.

Material and Methods

Experimental Site

The provenance-trial (EVENT 3-Landau) is a subproject of the EVENT-Experiment series in Bayreuth, Germany (Beierkuhnlein et al. 2011). It was established in 2010 in the vicinity of the University of Koblenz-Landau, at the Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Siebeldingen (49°13'03" N, 8°02'47" E, 202m a.s.l.). The mean annual temperature at the site is 10.2°C and the mean annual precipitation is 643mm, which is distributed bimodally with two major peaks in Mai/June and November/December (data: German Weather Service). Annual average temperature is expected to increase by 3.7K by the end of the century (2080s compared to 1950-2000), and precipitation is expected to decrease by about 50mm per year, with a major decrease in summer (JJA) precipitation (- 60mm), according to the A1B scenario of the MPI-ECHAM5-model (data: worldclim).

Plant Material

Seeds of six provenances of F. sylvatica were obtained in autumn 2009 and brought to germination at the Bavarian Institute for Forest Seeding and Planting (ASP) in Teisendorf, Germany in spring 2010. All six provenances stem from autochthonous populations (Fig. 1, Tab. 1). The provenances were chosen to represent the centre of the distribution range (DE1, DE2, DE3) and the south-eastern (BG) and south-western (ES) edges, where drought limits the distribution of the species, and the north-eastern edge (PL), where besides summer precipitation winter conditions and late frost are important limiting factors (Tab. 1, Fig. 1). In January 2011, the seedlings were transported to Siebeldingen and in March 2011, they were planted in 12-litre pots with two different soil types. Individuals were selected randomly for each provenance and treatment from all living plants at planting date. Mean plant height at the

start of the experiment was 22.1 cm (± 6.6cm SD), mean plant diameter 2cm above the root collar 4.7mm (4.4 – 5.3mm) with a standard deviation of ± 0.8mm SD (0.6-0.8mm).

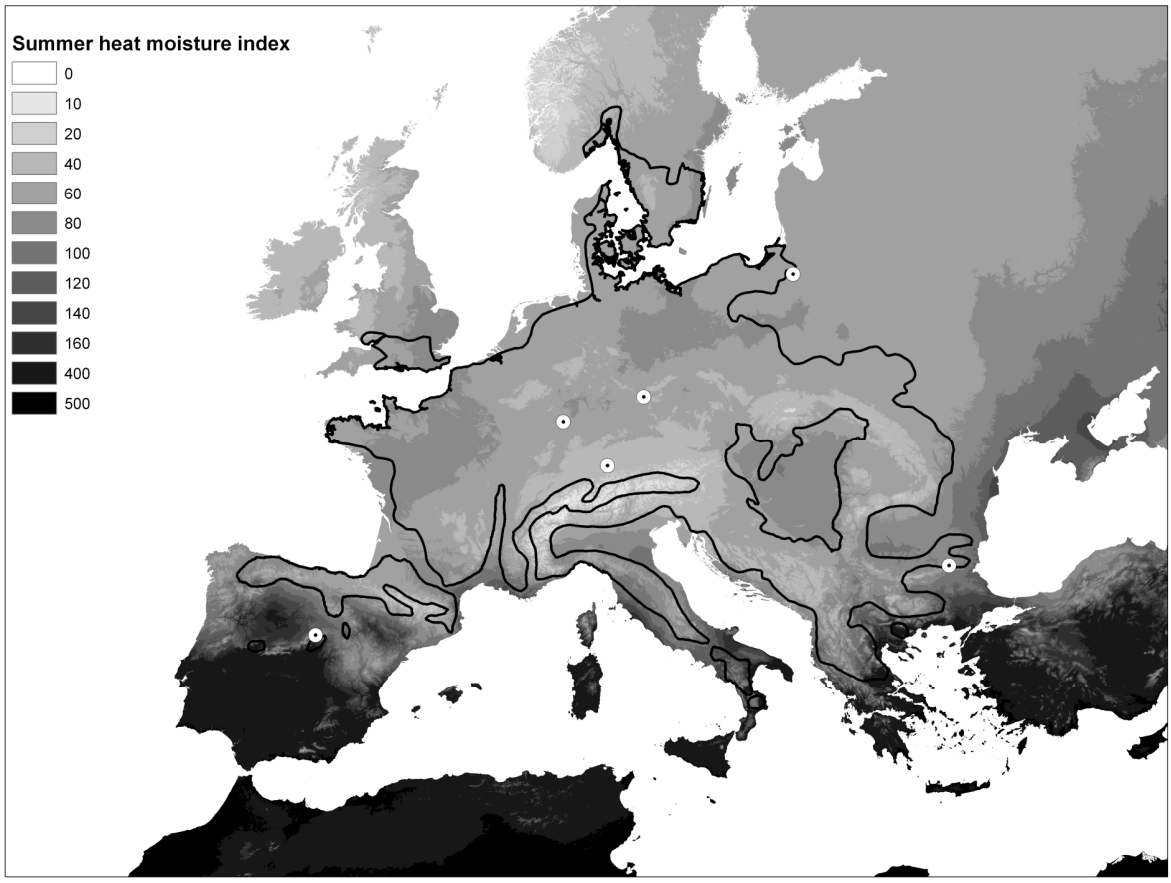

Figure 1: Geographic origins of provenances used in the experiment. Grey scales display the Summer Heat Moisture index based on worldclim data (Hijmans et al. 2005). Distribution range of *F. sylvatica* is outlined according to EUFORGEN

Table 1 Site information for the provenances used in the experiment. Summer Heat Moisture index derived from WorldClim (Hijmans et al. 2005).

Code	*Location*	*Country*	*Latitude*	*Longitude*	*elevation (m a.s.l.)*	*SHMI*
BG	Kotel	Bulgaria	N 42°51'59"	E 26°26'40"	600	61
ES	Montejo de la Sierra	Spain	N 42°01'00"	W 03°05'00"	1463	68
DE1	Hengstberg	Germany	N 50°08'00"	E 12°11'00"	569	47
DE2	Johanniskreuz	Germany	N 49°18'00"	E 07°50'00"	570	42
DE3	Kempten	Germany	N 47°44'48"	E 10°08'54"	803	26
PL	Mragowo	Polen	N 53°52'00"	E 21°20'00"	137	51

Experimental set-up

The potted individuals were exposed to a fully crossed experimental design consisting of a precipitation treatment (drought and control) and two different soil types (sandy loam, loamy sand). Each treatment combination (drought with sandy loam, drought with loamy sand, control with sandy loam and control with loamy sand) was replicated with 9 individuals. For two provenances (Spain, Poland) the number of replicates had to be reduced to 8 and 7 respectively, due to mice damage, resulting in 204 plants overall. The experiment was set up completely randomized in a concrete bed filled with sand. All pots were put on plastic saucer

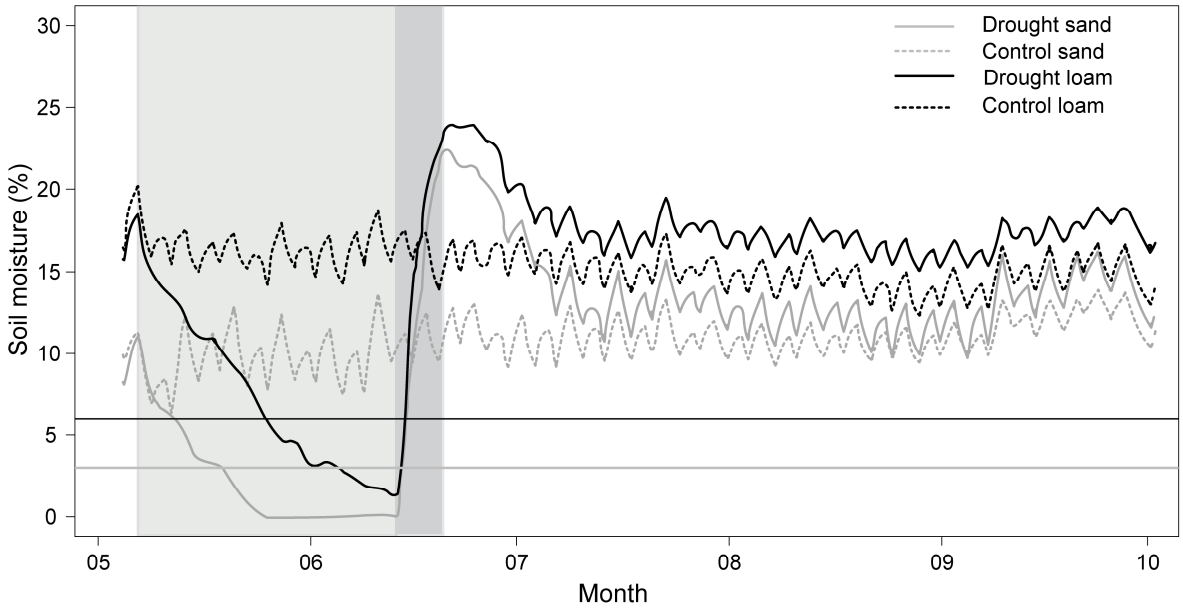

Figure 2: Soil moisture for all four treatment combinations. The light grey area shows the artificial drought period, the dark grey area shows the re-watering phase. The two horizontal lines depict the approximated permanent wilting points for the loamy (black) and the sandy substrate (grey).

On April 13th the experiment was covered by a rain-out shelter constructed of a steel frame (GlasMetall Riemer GmbH, Rahden-Sielhorst, Germany) and covered with a transparent polyethylene sheet (0.2mm, SPR 5; Hermann Meyer KG, Rellingen, Germany). The lower edge of the rain-out shelter was 80 cm above ground. The shelter permitted the penetration of nearly 90% of the photosynthetically active radiation. Additionally, as seedlings of F. sylvatica are sensitive to direct radiation, a shading canvas (Quadra 105ME, 105g, DM-Folien GmbH, Reutlingen, Germany) was attached to the inside of the polyethylene sheet, resulting in a penetration of about 55 % of the photosynthetically active radiation. As the shade-tolerant species European beech has shown to yield highest growth rates when exposed to medium light levels (e. g. Schall et al. 2012) most likely no limitation by light availability occurred in our experiment. From the planting date until the rain-out shelter was set up, the plants received the ambient precipitation and were additionally watered with groundwater (March

14th, 18th, April 1st, 4th 8th and 11th). After the rain-out-shelter was set up, the plants were watered with groundwater on April the 18th, 28th and 30th, so that a sufficient water supply and root growth was guaranteed. From May 2nd on all plants were exposed to the control precipitation regime which simulated the local daily 40-year average precipitation. The application was done twice a week with groundwater.

The drought manipulation consisted of a 36-day period without irrigation. It started on May 9th end was completed when 20% of the plants showed strong drought damage (76-100% of the leaves damaged), on June 13th. Approximately 12 days after the start of the treatment soil moisture dropped below the permanent wilting point (pF = 4.2; Fig.2) for the loamy sand substrate and about one week later for the sandy loam substrate. In the re-watering phase after the drought period the plants were exposed to the control precipitation treatment again. Additionally they received the amount of water which was given to the control plants over the 36-day drought period, evenly distributed over one week, so that at the end of the experiment both the individuals in the control as well as in the drought treatment received the same amount of water.

The volumetric soil water content was measured with dielectric permittivity sensors (EC-5 soil water sensor, Decagon Devices, Inc., Pullman, USA). Altogether 10 sensors with two data loggers (ECH2O, Decagon Devices, Inc. Pullman, USA) were installed in the pots in 5-10 cm depth, three for the two soil types each in the drought treatment and two each in the control treatment.

The soil treatment consisted of two different soil types. The first one was a sandy loam substrate from a local forest top-soil dug-out, from now on called loam or loamy substrate.

The second soil type was a loamy sand substrate, consisting of a mixture between the loam and arenaceous quartz sand from a local sand pit (50% each), from now on referred to as sand or sandy substrate (Tab.2).

Table 2 Nutrient content and pH-values fort he two soil types used in the experiment

	K ***mg/kg***	***Mg*** ***mg/kg***	***P*** ***mg/kg***	***NO3*** ***mg/kg***	***NH4*** ***mg/kg***	***Corg.*** ***%***	***pH-CAT***	***pH-water***	***N*** ***%***
Loamy **Sand**	54,8	121	11,1	14,1	2,02	0,56	6,3	8,5	<
Sandy **Loam**	118	267	48,3	31,0	3,98	1,92	4,7	7,6	0,14

Response Parameters

Short-term response to the treatments was determined by visual estimates by assigning the average percentage of damaged leaves per plant to six classes (0%, 1-5%, 6-25%, 26-50%, 51-75%, 76%-100%). A leave was counted as "damaged", as soon as it exhibited necrotic tissue. The share of necrotic tissue of the whole leave was not taken into consideration. This assessment took place at the last day of the drought treatment. For each individual, mid points of the six classes were used for further statistical analyses.

Long-term effects of the treatments were determined by measuring height and diameter shortly after planting time in April and after the growing period at the end of September. Increment was calculated by subtracting the initial values from the values at the end of the growing period. The statistic were conducted both for absolute and relative increment values. As the initial plant dimensions were very similar over the provenances and thus the relative values yielded similar results we concentrate on absolute values in this study. Dead individuals were counted and the percentage of dead individuals per treatment, soil type and provenance was calculated. Furthermore, the Summer Heat Moisture Index (Wang et al. 2006) was calculated ((mean temperature of warmest month)/(mean annual summer (May-September) precipitation/1000)) for each geographic origin of the provenances (data: WorldClim; Hijmans et al. 2005).

Statistics

Analyses of Variance (ANOVA) combined with linear models were applied to test for the main and interactive effects of the three factors provenance, drought and soil on height and diameter increment and on leaf damage. Data was square-root transformed in order to improve homogeneity of variance and normality of residuals prior to analysis if necessary. In case of significant effects, post hoc comparisons were run (Tukey's HSD).

To test for the long term impacts of short term damage, an ordinary least squares regression was applied between the reduction of height and diameter increment in the drought treatment compared to control (in %) and the percentage of damaged leaves per plant averaged per provenance. Generalized Linear Models were used to determine the effects of treatment, soil and provenance on mortality, Least Squares Regressions to correlate mortality with increment rates.

Least squares regressions were also used to assess the correlation between the Summer Heat Moisture Index (SHM) at the geographic origins of the provenances and increment reduction

(drought compared to control in %), leaf damage (%) and mortality (%) in order to detect local adaptations to drought events.

All statistical analyses were conducted with the software R.2.13.0 (R Development Core Team 2011).

Results

Leaf damage

The provenances differed in the percentage of damaged leaves per plant (ANOVA: F = 2.5, p = 0.035; Fig. 3a). The German provenance from Kempten (DE3) showed the highest share of damaged leaves (23%), whereas the Bulgarian provenance exhibited a significantly lower percentage of damaged leaves (6.5%). The drought treatment increased the percentage of damaged leaves significantly from 4% in the control treatment to 26% on average for plants under the drought treatment (ANOVA: F = 30.9, p < 0.001; Fig. 3b). Plants grown in sand showed a significantly higher share of damaged leaves (22.1%) than plants in loam (7.8%) (ANOVA: F= 16.1, p < 0.001; Fig. 3c).

Soil type significantly impacted the drought effect on leaf damage (ANOVA: soil x drought: F = 9.0, p = 0.003; Fig. 3d). In the loamy substrate drought caused 4.7 times (D: 12.8%, C: 2.7%) more damage compared to control, whereas in sand drought caused 7.2 times (D: 39.1%, C: 5.4%) more damage than the control treatment. Provenance-specific reactions to drought or soil type could not be detected (ANOVA: provenance x drought: F = 1.3, p = 0.283; provenance x soil: F = 2.3, p = 0.128).

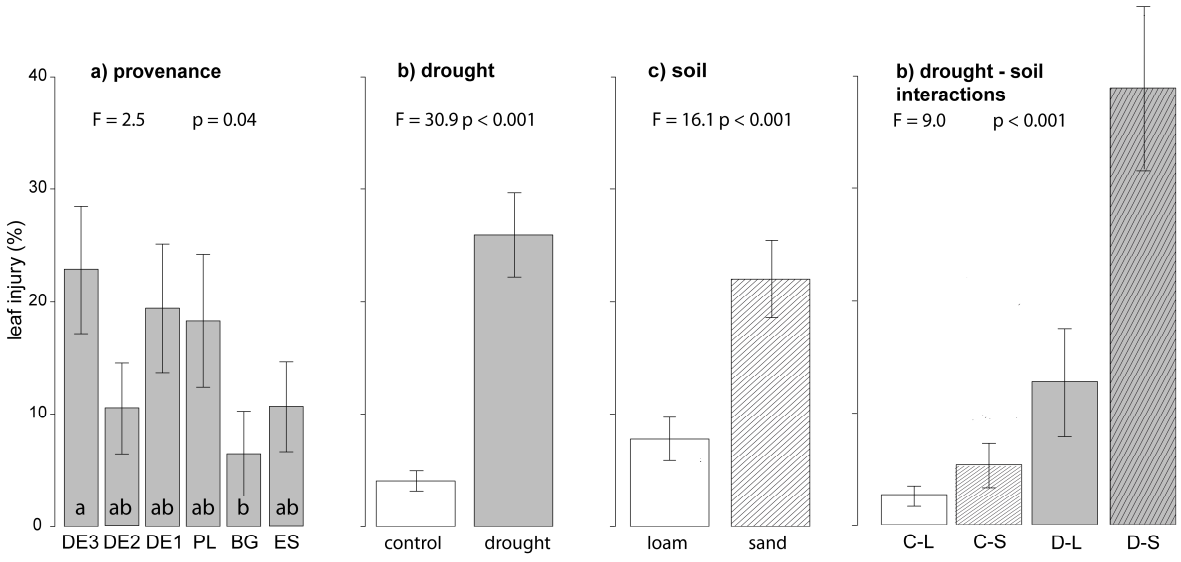

Figure 3: Leaf damage of *F. sylvatica* at the end of the drought manipulation (June 13th) in % in response to a) provenance, b) drought and c) soil type. All significant main and interaction effects (d) of the three-factorial ANOVA are displayed. Provenances are arranged with increasing SHMI

Height increment

Provenances differed in terms of height increment (ANOVA: $F = 18.5$, $p < 0.001$; Fig. 4a). The Bulgarian and the German provenance from Johanniskreuz (DE2) showed significantly less increment than the other provenances (BG: 5.1cm, DE2: 5.7cm). The German provenance Hengstberg (DE1) performed best with 14.9cm height increment. The drought treatment reduced the height increment significantly from 13.5cm under control conditions to 8.1cm on average for plants exposed to drought conditions (ANOVA: $F = 32.9$, $p < 0.001$; Fig. 4b). Soil did not influence increment in height (ANOVA: $F = 2.1$, $p = 0.149$; Fig. 4c).

The provenances showed different height increment patterns across the two soil types (ANOVA: provenance x soil: $F = 2.6$, $p = 0.026$; Fig. 4d). The Bulgarian provenance reacted positively to sandy soil, whereas all other provenances showed no or a negative reaction to the sand compared to the loam.

Provenance-specific reactions to drought or interactive effects of drought and soil could not be detected (ANOVA: provenance x drought: $F = 1.4$, $p = 0.235$; drought x soil: $F = 2.3$, $p = 0.132$).

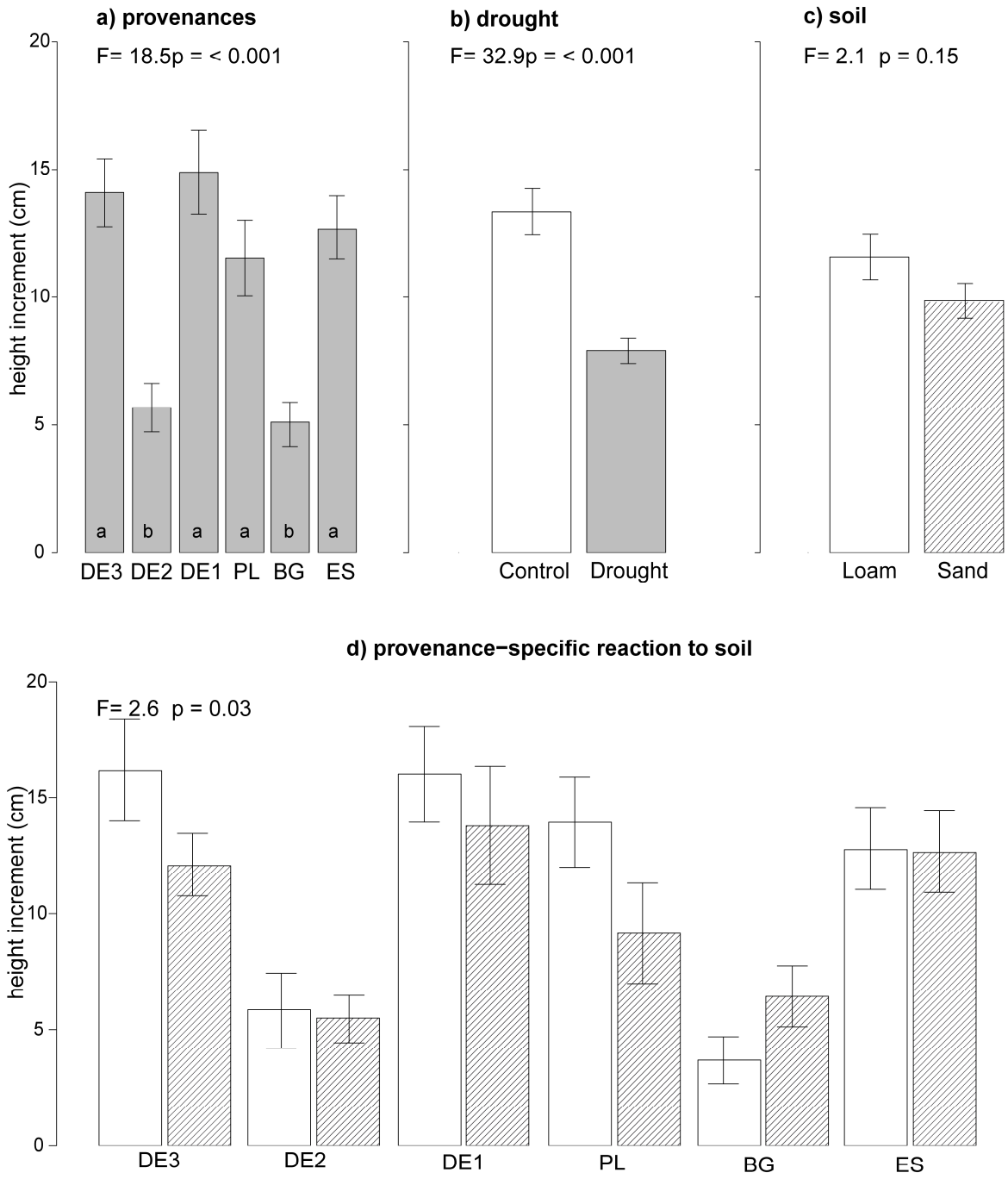

Figure 4: Height increment of *F. sylvatica* in response to a) provenance, b) drought and c) soil type. All significant main and interaction effects (d) of the three-factorial ANOVA are displayed. Provenances are arranged with increasing SHMI

Diameter increment

The Bulgarian provenance (BG) grew significantly worse (2.0mm) than all other provenances in terms of diameter (ANOVA: F = 3.1, p = 0.011, Fig. 5a), while all others showed similar growth rates (2.6-2-9mm). The drought treatment reduced the diameter increment significantly from 3.4mm under control conditions to 2.0mm on average for plants exposed to

drought conditions (ANOVA: $F = 89.7$, $p > 0.001$, Fig. 5b). The sandy substrate also had negative impacts on secondary growth, reducing increment from 3.1mm in loamy substrate to 2.2mm in sandy substrate (ANOVA: $F = 37.3$, $p < 0.001$, Fig. 5c).

The two soil types triggered a provenance-specific reaction in terms of diameter increment (ANOVA: provenance x soil: $F = 2.6$, $p = 0.027$; Fig. 5d). The Bulgarian and Spanish provenances responded with a relatively stable diameter increment to sandy substrate compared to the loam, whereas all other provenances showed higher growth rates on loam. The German provenance Hengstberg (DE1) exhibited a notably strong decline in mean diameter increment on sand (even if not significantly different from reactions of other German and Polish provenances). Furthermore the provenances showed a different tolerance to drought with regard to diameter increment (ANOVA: provenance x drought: $F = 2.7$, $p = 0.020$, Fig. 5e). Only a minor decrease could be detected for the Bulgarian provenance, while the German provenances Hengstberg (DE1) and Kempten (DE3) showed strong cutbacks in diameter increment in response to drought. Soil type significantly impacted diameter increment in response to drought (ANOVA: drought x soil: $F = 7.7$, $p = 0.006$). The sand fostered the negative impacts of the drought period on diameter increment (control: 3.1mm; drought: 1.3mm), whereas in the loamy soil the drought impact was more moderate (control: 3.6mm; drought: 2.6mm).

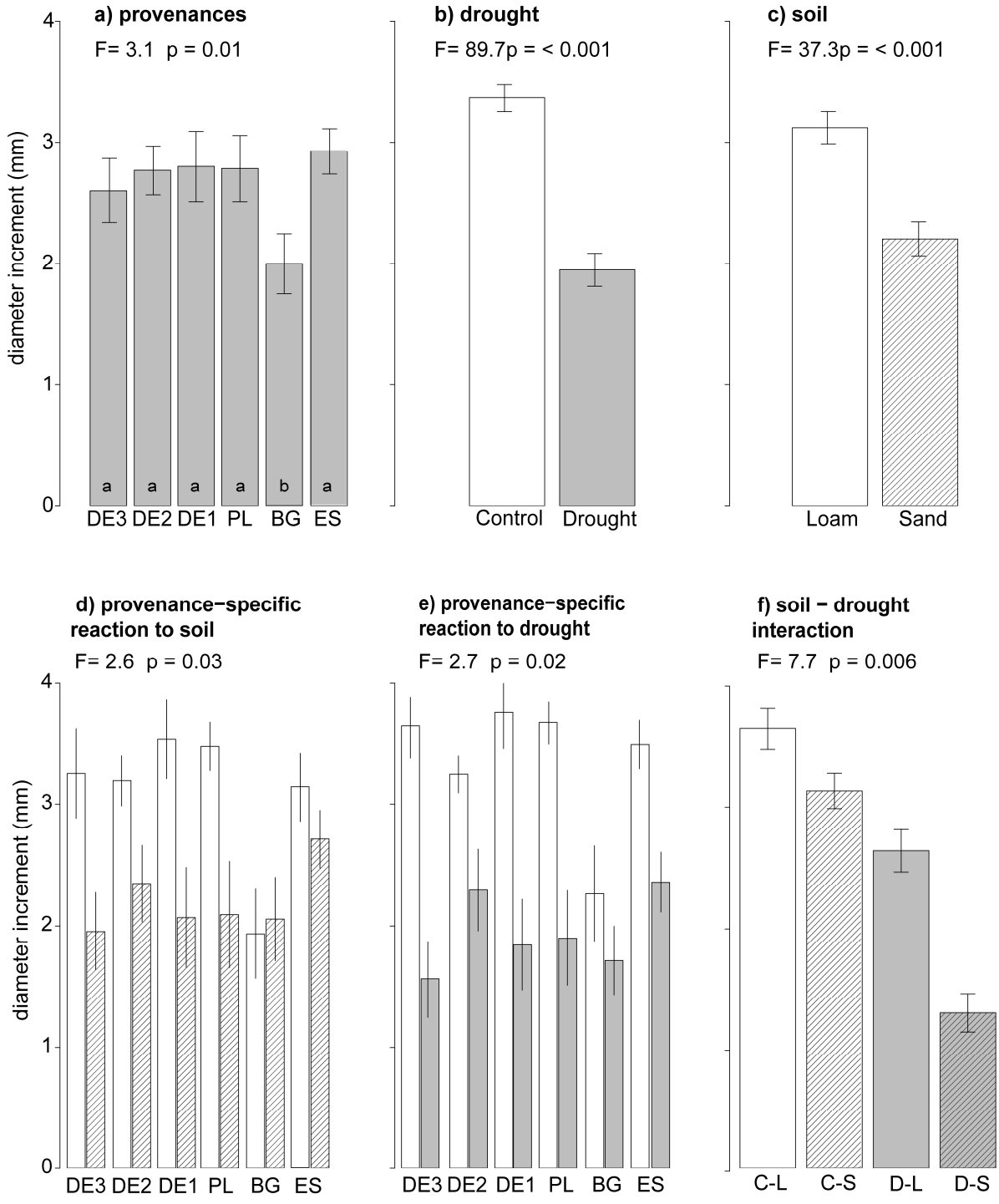

Figure 5: Diameter increment of *F. sylvatica* in response to a) provenance, b) drought and c) soil type. All significant main and interaction effects (d-f) of the three-factorial ANOVA are displayed. Provenances are arranged with increasing SHMI.

Mortality

Mortality occurred solely within the drought treatment, and within the drought treatment mainly in the sandy substrate (drought/loam = 7.8%, drought/sand = 25.5%; ANOVA: p =

0.012). Provenance did not impact mortality significantly (ANOVA: $p = 0.097$), even though mortality correlated significantly with summer drought conditions at the places of origin of the provenances (see Results, Local adaptation). Mortality tended to increase (non – significantly) with diameter increment reduction (adjusted $r^2 = 0.39$, $p = 0.110$) in the drought treatment compared to control.

Correlation of leaf damage with increment

The average percentage of damaged leaves per provenance was strongly correlated to a reduction in diameter increment (adjusted $r^2 = 0.99$, $p < 0.001$). The reduction in height increment exhibited a non-significant trend towards an increasing reduction with increasing leaf damage (adjusted $r^2 = 0.42$, $p = 0.097$).

Local adaptation

The Summer Heat Moisture index (SHM) at the geographic origin of the provenances did not influence the reaction to drought with regard to height increment (adjusted $r^2 = 0.01$, Fig. 6a). However, even if non-significant, provenances from origins which are more prone to summer drought events (higher SHM) tended to be more drought tolerant in terms of diameter increment and leaf damage, as SMH at the geographic origins explained 25% of the variance in diameter increment reduction (Fig. 6b), 31% of the variance in leaf damage (Fig. 6c). A significant correlation ($p = 0.041$) could be detected between the SMH index and mortality (Fig. 6d). Here the summer heat moisture index at the geographic origin explains 69% of the variance in mortality.

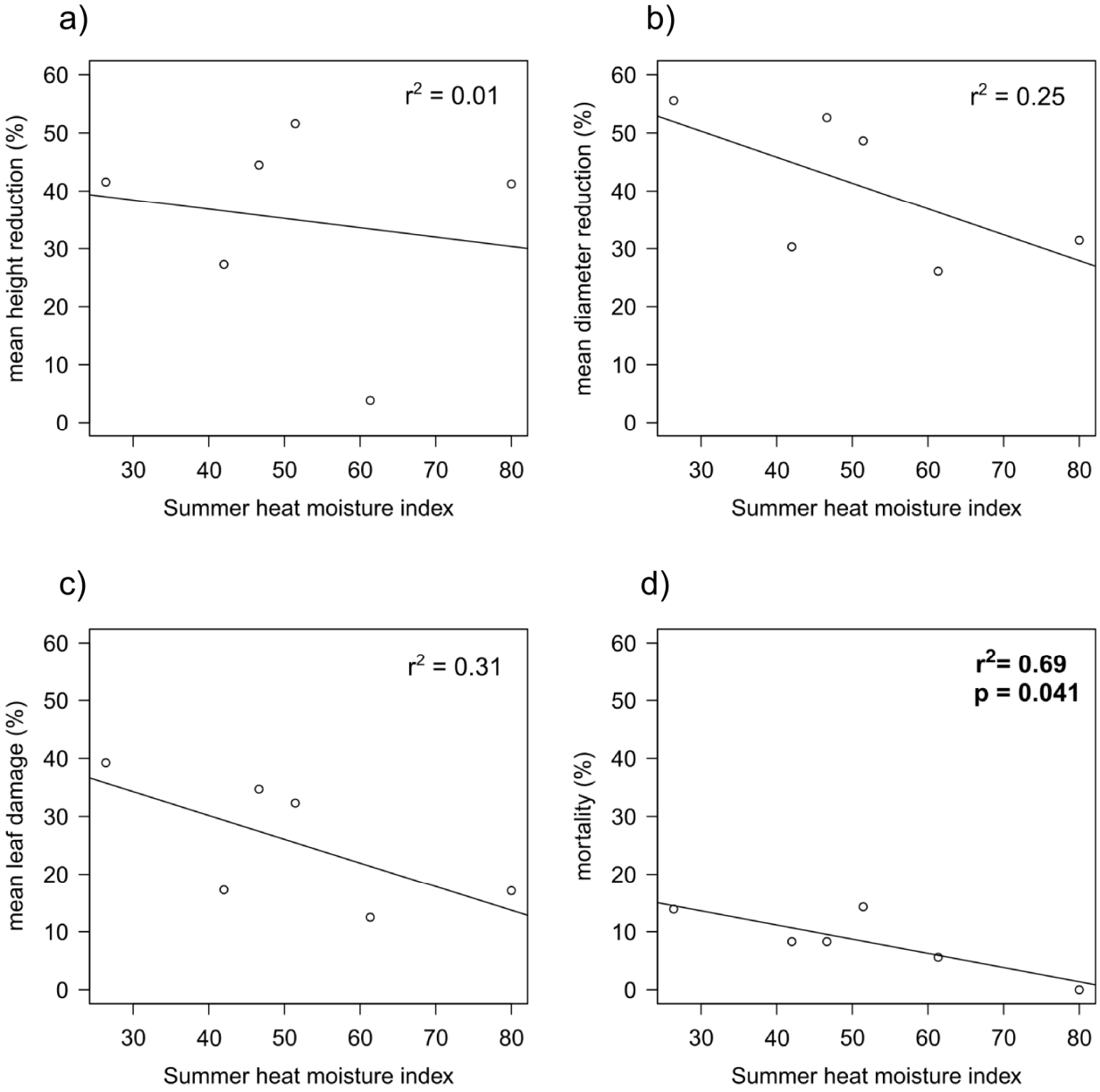

Figure 6: Correlation between the summer heat moisture index at the origin of the provenances and the mean reduction in a) height and b) diameter increment, c) mean leaf damage per provenance and d) mortality per provenance.

Discussion

Intra-specific variation

The current distribution of F. sylvatica is a result of multiple glacial periods. F. sylvatica survived the last glacial maximum in several refuge areas in Europe (Magri et al. 2006). Most probably, refuge areas in the South-Western and Dinaric Alps played an important role in the re-colonization of Central- and Western Europe, whereas the Mediterranean populations seem to have survived in several geographically distinct refuges on the Iberian and Italian

peninsulas and in the Balkan (Magri et al. 2006, Magri 2008, Brus 2010). In particular the Balkan (and Italian) populations are genetically distinct from the other European populations (Magri 2008). This post-glacial migration pattern and genetic differentiation, most probably, also resulted in phenotypic variation between provenances. Distinct performances of European beech provenances grown in common garden experiments are well-known and often demonstrated (e.g. von Wuehlisch et al. 1995, Nielsen and Jorgensen 2003, Giannini and von Wuehlisch, 2009). The differences between provenances in all of the examined parameters in this study are consistent with these previous findings and likely express the differences in genetic make-up.

Especially the Bulgarian provenance showed a distinct performance in all parameters. Bulgarian beech populations are genetically distinct from Central European populations (Magri et al. 2006), which may explain the significantly lower increment. Surprisingly, one German provenance (DE2) performed significantly worse in terms of height increment than the other two German provenances (DE1, DE3), despite a most likely similar post-glacial history and assumingly comparable genetic make-up. Yet, in Central Europe genetic diversity is higher within populations than between populations (Konnert 1995). Furthermore, the silvicultural history of particular beech stands is often hard to be reconstructed. In addition, local soil conditions and small-scale rainfall-patterns may be also responsible (see below).

Effects of drought and soil on tree performance

Drought adversely impacted all measured parameter significantly: It increased the amount of leaf damage and decreased height and diameter increment. This is in compliance with numerous studies reporting on the drought sensitivity of F. sylvatica (Rennenberg et al. 2004, Lebourgeois et al. 2005, Jump et al. 2006, Piovesan et al. 2008, Friedrichs et al. 2009, Betsch et al. 2011, Hlasny et al. 2011, Jezik et al. 2011, Scherrer et al. 2011).

As expected, the sandy substrate with lower nutrient availability and water storage capacity resulted in more severe negative impacts on the performance of plants, compared with loamy substrate. The drought impact on leaf injury rates and diameter increment was more severe for plants grown in the sandy substrate, which shows the importance of the interaction between precipitation rates and soil properties (Gaertner et al. 2008). The soil moisture in the sandy substrate dropped below the approximated permanent wilting point one week before this point was reached in the loamy substrate. Plants in the sandy substrate, therefore experienced ~24 days of water stress, whereas plants grown in the loamy substrate only ~17 days. The reduced

nutrient uptake due to lacking water absorption during the drought might furthermore have weaker impacts in nutrient-richer soils than in poorer soils. Apart from nutrient uptake, evidence suggests that also nutrient availability of the soil is reduced due to drought conditions (Rennenberg et al. 2009, Kreuzwieser and Gessler 2010).

In this study we did not investigate biomass allocation to above- and belowground components of the tree seedlings. Especially at a seedling age, the ability of European beech to plastically react to environmental/drought conditions in terms of reallocation of resources is quite high, as Schall et al. (2012) demonstrated. European beech seedlings increased the investment into belowground biomass as a reaction to drought. According to the balanced-growth hypothesis (Hunt 1975, Shipley and Meziane 2002) plants reallocate resources to the organ that exploits the limiting resource, in our case the root system. Such potential reallocation effect of a drought treatment might lead to over-estimation of growth reduction, as it might mask a constant biomass production, when just observing above ground-growth. When trees get older this morphological plasticity decreases and physiological acclimatization processes becomes more important (Schall et al. 2012). Therefore a transfer of results from seedlings to mature trees should be backed by more research, e.g. in long-term provenance trials.

Provenance-specific reactions and local adaptation to drought and soil

Drought-sensitivity in terms of diameter increment varied between the provenances in our experiment. The Bulgarian provenance (BG) showed a weak performance under the control treatment, yet the smallest reduction of diameter increment due to the drought treatment. The increment reduction between drought and control in other provenances (DE1, DE3, PL) amounted to 50%. However, their absolute increment values under drought conditions were still higher than for the BG provenance. This behaviour of provenances correlates strongly with leaf injury rates ($r^2 = 0.99$). The interactive effect of provenance and drought treatment was not significant in this case, yet this is due to the fact that leaf injury almost exclusively occurred under the drought treatment. Given the relatively small number of provenances in our trial, significant correlations between the drought response of increment and leaf damage with the climate at the origin of the provenances could not be established. Yet, the reactions of both parameters tended towards a correlation with the long-term summer heat moisture index at the origin of the provenances. This trend is supported by previous findings by Czajkowski and Bolte (2006) who demonstrate a correlation between the drier, continental

climate at the origin of a beech provenance from central Poland with low water stress sensitivity and the moister climate of provenances from Germany and West Poland with higher drought stress sensitivity. It is further in compliance with the results of Nielsen and Jorgensen (2003) who found a significant interaction between soil water content and diameter increment for 14 provenances in a common garden experiment in Denmark. However, these authors found a greater adaptability of southern provenances to changing water conditions. In our experiment, the Bulgarian provenance showed a low but stable increment over the treatments while most northern provenances (except DE2 Johanniskreuz) performed at similar low levels under drought conditions but showed a much more positive response to favourable conditions than the Bulgarian one. Similar to the Bulgarian provenance the Spanish one is not very responsive to drought in terms of leaf damage under dry conditions and showed the lowest mortality rates, however, in contrast to the Bulgarian provenance on a higher increment level and more notable reductions in increment. Surprisingly the performance of the German provenance from Johanniskreuz (DE2) resembles the more the southern provenances (than the other two German and the Polish provenances, which are geographically closer) with low leaf injury rates, low height growth and lower drought induced growth reductions. Nielsen and Jorgensen (2003) show in their study that northern provenances display a low but stable growth level and southern ones a more plastic response.

The results of our study and the results of Nielsen and Jorgensen might be superficially interpreted as inconsistent. On closer inspection, however, the mere geographic division of provenances into North and South might not reflect the actual climatic and site conditions populations were exposed to in past and present at their places of origin. The southern provenances used by Nielsen and Jorgensen (2003) do not stem from the very edges of the distribution range, except one from Mount Etna, Sicily (IT) and the places of origin of these southern provenances have relatively high precipitation rates, whereas the northern provenances come from regions with relatively continental climate with low precipitation sums (e.g. eastern Germany and Central Poland). They might be even more exposed to dry conditions at their origins. The same might be applicable for the concept of geographically marginal and central populations, as this concept excludes micro- and mesoclimatic effects, and small-scale differences in soil and site conditions. Even in the centre of the distribution range, very unfavourable and ‘marginal’ sites can be found, whereas on the geographic margins favourable growth conditions can occur on small scale, e.g. at the wind-ward side of small mountain ranges with orographic rainfall-patterns.

In this study the summer heat moisture index of the ES provenance is even higher than for BG, still the drought-tolerance in term of stable growth is not as high as in BG, which may be due to edaphic reasons (Gaertner et al. 2008). This could mean that the actual drought severity, derived from precipitation and soil characteristics, might be lower at the origin of the Spanish provenance (ES). The behaviour of the German provenance (DE2) is also similar to the marginal provenance from BG, although it stems from the centre of the distribution range with climatic conditions comparable to the other German provenances. DE2 stems from the Palatinate Forest, a German mountain range with lower Triassic sandstone formations. The sandy soil there might create much drier conditions than what the other two German provenances experience at their point of origin. Furthermore information on slope, exposition, soil depth and substrate at the exact location of seed sampling (i.e. of single mother trees) might improve explanatory power in future analyses.

Additionally, these intricate findings might indicate a complex pattern of within and between population variations, developed from the interplay of post-glacial history, local climatic adaptation, local growing conditions and human silvicultural practices. Herbette et al. (2010) and Wortemann et al. (2011) claim that phenotypic variability in drought tolerance, here measured in cavitation resistance, is mainly caused by phenotypic plasticity and not by genotypic differences between populations. Yet, our data point towards some degree of inherited local adaptation to climate conditions at the origins of the provenances even when considering our small selection of provenances. Accordingly, marginal beech populations which face more adverse conditions are under stronger genetic selection (Wortemann et al. 2011) than populations on favourable sites. In our study, the Bulgarian provenance (BG) shows the most distinct provenance effect and provenance-specific reaction. It stems from the very south-eastern distribution edge of F. sylvatica, where summer drought is most probably the range-limiting factor (Jump et al. 2006). This again emphasizes the importance of marginal beech populations in the search of drought resistant ecotypes (Rose et al. 2009).

A trade-off between survival under drought conditions and high increment under favourable conditions has been shown for four broad-leaved species in Kenya (Kondoh et al. 2006). Accordingly, F. sylvatica displays the lowest mortality under shade, yet the lowest growth rate under light compared to other deciduous trees (Petritan et al. 2007). Likewise, a continental and a Mediterranean Pinus sylvestris provenance did not differ under dry conditions, whereas under wetter conditions the continental provenance showed higher root biomass allocation and seedling recruitment rates (Richter et al. 2012). This confirms

previous findings, that high phenotypic plasticity appears beneficial in highly variable environments.

The drought manipulation in our experiment can be considered as relatively mild. Only few individuals died due to the treatment. Provenance did not impact the mortality rate significantly in general. However, there is a clear and significant correlation between summer drought and mortality, with lower mortality rates of the southern marginal provenances due to drought treatment (BG = 11.1%, ES = 0%, all provenances 15.3%), providing evidence for an adaptation to local climate conditions. Given the above mentioned potential trade-off mechanisms this slight trend towards higher mortality with stronger diameter growth reduction, could point towards higher survival rates of less plastically responding provenances. A stronger genetic selection in drought-prone ecotypes (Wortemann et al. 2011) could lead to lower genetic diversity (Kawecki 2008) and thus low phenotypic plasticity (Schaberg et al. 2008, Matyas et al. 2009), though Kawecki (2008) also reports that for some marginal species no genetic depauperation for ecologically relevant traits could be detected. Therefore marginal populations could potentially secure ecosystem persistence under very extreme climatic events, yet might increase the risk of lower growth rates under favourable conditions.

Nagy et al.(in prep.) found a similar pattern in a study in which Bulgarian and German Beech provenances were exposed to drought conditions, with low growth, but stable reaction to drought in some provenances and higher growth rates, but strong reduction for other provenances. Yet, the reaction did not depend on the origin of the provenance.

Furthermore, the Bulgarian, Spanish and the German provenance from Johanniskreuz (DE2) showed relatively stable responses in height and diameter increment in the sandy substrate compared to the loamy substrate. This might indicate that these provenances are not able to exploit the improved water and nutrient condition in the loam. It could also indicate a local adaptation to certain soil characteristics, which was e.g. found for Pinus nigra (Varelides et al. 2001). Still, such a potential trade-off between stress-tolerance and high performance under more favorable conditions is yet to be supported by more evidence in future provenance trials.

At the north-eastern edge of the distribution range the SHM is not as high as at the southern edge. The Polish provenance (PL) originates from the ecotone between beech dominated forest and boreal forests. Beech dominates on moraine loamy soil, while Scots pine dominates on drier and sandier soils (Bolte et al. 2007). Especially at the north-eastern margin where continental climate conditions are becoming more prevailing correlations with single macro-

climatic factors fail to explain distribution margins of F. sylvatica (Bolte et al. 2007). There, winter conditions and frost are at least as important as drought. Giving the clear response to drought in our experiment it seems that a strong selection towards drought-resistant ecotypes does not take place.

Like mentioned above, below-ground biomass production was not determined in this study, yet there might be differences in plasticity of biomass allocation between the provenances (Richter et al. 2012), which deserve more attention in future studies.

Conclusion and implications for research and forest management

Provenances of F. sylvatica differ in increment and exhibit differences in their sensitivity to drought. This intraspecific variability can probably be used to adapt forest ecosystems to future climate conditions. Yet, the impact of the interplay between climatic and soil and site characteristics on the emergence of drought conditions have to be accounted for as provenances react specifically to contrasting soil types. Therefore the concept of marginality should be extended from a geographical to a more site-related concept; yet, especially marginal populations from the drought-prone southern edges of the distribution range are potential targets in the search for drought-resistant ecotypes. However, a potential trade-off between high phenotypic plasticity with high performance rates under favourable conditions, and stress-tolerance and survival under drought conditions and its impact on forest growth requires future research. Long-term provenance trials with a greater number of central and marginal (maybe also from 'marginal' sites in the centre of the distribution range) provenances, where the performance of trees can be monitored beyond the seedling age, could contribute in approaching these open questions. Differing from existing trials, a hypothesis-based selection of provenances and the option for climate manipulations in later ontogenetic stages should be applied. Furthermore, a single drought manipulation does not reflect a change in long-term mean values and changes in frequency of extreme events and there long-term impacts. Therefore, a network of several climatically different trial sites, including marginal sites is needed.

Furthermore, more multi-factorial experiments could address the problem that resistance to one abiotic factor such as drought does not necessarily imply resistance to other climatic or abiotic factors such as frost (Kreyling et al. 2012) or forest pests. However, the establishment of multi-factorial, multi-site and long-term trials and experiments will take longer than there is time to start adaptive actions in forest management with regard to the velocity of climate

change. Evidence suggests that the selection of a single best drought-adapted provenance for transplantations is not reasonable, considering the limited knowledge of the long-term effects. Nonetheless, marginal and drought-adapted ecotypes might be intermixed, together with local provenances and ecotypes adapted to other abiotic factors, in order to secure yield and stability under more extreme climatic conditions in the future. Forest management should therefore aim at increasing the genetic diversity of forest stands to secure populations against breakdown due to climatic extremes.

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References

Allen CD, Macalady AK, Chenchouni H et al (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manag 259:660–684

Beierkuhnlein C, Thiel D, Jentsch A et al (2011) Ecotypes of European grass species respond differently to warming and extreme drought. J Ecol 99:703–713

Bertrand R, Lenoir J, Piedallu C et al (2011) Changes in plant community composition lag behind climate warming in lowland forests. Nature 479 (7374):517-520

Betsch P, Bonal D, Breda N et al (2011) Drought effects on water relations in beech: The contribution of exchangeable water reservoirs. Agric For Meteorol 151:531–543

Bolte A, Czajkowski T, Kompa T (2007) The north-eastern distribution range of European beech - a review. Forestry 80:413–429

Bolte A, Hilbrig L, Grundmann B et al (2010) Climate change impacts on stand structure and competitive interactions in a southern Swedish spruce-beech forest. Eur J For Res 129:261–276

Bolte A, Ammer C, Lof M et al (2009) Adaptive forest management in central Europe: Climate change impacts, strategies and integrative concept. Scand J For Res 24:473–482

Bonn S (2000) Competition dynamics in mixed beech-oak stands and its modifications expected due to climate changes. Allg For Jgdztg 171:81–88

Brus R (2010) Growing evidence for the existence of glacial refugia of European beech (*Fagus sylvatica L.*) in the south-eastern Alps and north-western Dinaric Alps. Period Biol 112:239–246

Charru M, Seynave I, Morneau F et al (2010) Recent changes in forest productivity: An analysis of national forest inventory data for common beech (*Fagus sylvatica L.*) in north-eastern France. For Ecol Manag 260:864–874

Chen I-C, Hill JK, Ohlemueller R et al (2011) Rapid Range Shifts of Species Associated with High Levels of Climate Warming. Science 333:1024-1026

Chiarucci A, Araujo MB, Decocq G et al (2010) The concept of potential natural negetation: an epitaph? J Veg Sci 21:1172-1178

Clark JS, Bell DM, Hersh MH et al (2011) Climate change vulnerability of forest biodiversity: climate and competition tracking of demographic rates. Global Change Biol 17:1834–1849

Comps B, Thiebaut B, Paule L et al (1990) Allozymic variability in beechwoods (*Fagus sylvatica* L.) over Central-Europe - Spatial differentiation among and within populations. Heredity 65:407–417

Czajkowski T, Bolte A (2006) Different reaction of beech (*Fagus sylvatica L.*) provenances from Germany and Poland to drought. Allg For Jgdztg 177:30–40

Czajkowski T, Kuhling M, Bolte A (2005) Impact of the 2003 summer drought on growth of beech sapling natural regeneration (*Fagus sylvatica L.*) in north-eastern Central Europe. Allg For Jgdztg 176:133–143

Czucz B, Galhidy L, Matyas C (2011) Present and forecasted xeric climatic limits of beech and sessile oak distribution at low altitudes in Central Europe. Ann For Sci 68:99–108

Dittmar C, Elling W (1999) Radial growth of Norway spruce and European beech in relation to weather and altitude. Forstw Cbl 118: 251-270

Ellenberg H, Leuschner C (2010) Vegetation Mitteleuropas mit den Alpen in ökologischer, dynamischer und historischer Sicht. 6th edition. Ulmer, Stuttgart

Fotelli MN, Gessler A, Peuke AD et al (2001) Drought affects the competitive interactions between *Fagus sylvatica* seedlings and an early successional species, *Rubus fruticosus*: responses of growth, water status and delta C-13 composition. New Phytol 151:427–435

Fotelli MN, Nahm M, Radoglou K et al (2009) Seasonal and interannual ecophysiological responses of beech (*Fagus sylvatica*) at its south-eastern distribution limit in Europe. For Ecol Manag 257:1157–1164

Friedrichs DA, Trouet V, Buentgen U et al (2009) Species-specific climate sensitivity of tree growth in Central-West Germany. Trees Struct Funct 23:729–739

Gaertner S, Reif A, Xystrakis F et al (2008) The drought tolerance limit of *Fagus sylvatica* forest on limestone in southwestern Germany. J Veg Sci 19:757–768

Gessler A, Keitel C, Kreuzwieser J et al (2007) Potential risks for European beech (*Fagus sylvatica L.*) in a changing climate. Trees 21:1–11

Giannini R, von Wühlisch G (2009) Workshop COST E52 “Evaluation of beech genetic resources for sustainable forestry”. iForest - Biogeosciences and Forestry 3:104.

Herbette S, Wortemann R, Awad H et al (2010) Insights into xylem vulnerability to cavitation in *Fagus sylvatica L.*: phenotypic and environmental sources of variability. Tree Physiol 30:1448–1455

Hijmans RJ, Cameron SE, Parra JL, Jones PG and Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Clim 25: 1965-1978.

Hlasny T, Barcza Z, Fabrika M et al (2011) Climate change impacts on growth and carbon balance of forests in Central Europe. Clim Res 47:219–236

IPCC (2012) Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change (ed. Field, C.B., Barros, V., Stocker, T.F., Quin, D., Dokken, D.J., Ebi, K.L., Mastrandrea, M., Mach, K., Plattner, G.-K., Allen, S., Tignor, M. & Midgley, P.). Cambridge Univ. Press, Cambridge, UK, pp. 1–19.

Jezik M, Blazenec M, Strelcova K et al (2011) The impact of the 2003-2008 weather variability on intra-annual stem diameter changes of beech trees at a submontane site in central Slovakia. Dendrochronologia 29:227–235

Jump AS, Hunt JM, Penuelas J (2006) Rapid climate change-related growth decline at the southern range edge of Fagus sylvatica. Global Change Biol 12:2163–2174

Jung T (2009) Beech decline in Central Europe driven by the interaction between *Phytophthora* infections and climatic extremes. Forest Pathol 39:73–94

Kawecki, T.J. (2008) Adaptation to marginal habitats. Annl Rev Ecol Evol System, 39, 321–342.

Kölling C. (2007) Klimahüllen für 27 Waldbaumarten. AFZ Der Wald:1242–1245

Kondoh S, Yahata H, Nakashizuka T, Kondoh M (2006) Interspecific variation in vessel size, growth and drought tolerance of broad-leaved trees in semi-arid regions of Kenya. Tree Physiol 26:899-904

Konnert M (1995) Investigations on the genetic variation of beech (*Fagus sylvatica L*) in Bavaria. Silvae Genet 44:346–351

Kreuzwieser J, Gessler A (2010) Global climate change and tree nutrition: influence of water availability. Tree Physiol 30:1221–1234

Kreyling J, Thiel D, Nagy L et al (2012) Late frost sensitivity of juvenile *Fagus sylvatica* L. differs between southern Germany and Bulgaria and depends on preceding air temperature. Eur J For Res: 131: 717-725.

Kreyling J, Bittner T, Jaeschke A et al (2011) Assisted colonization: A question of focal units and recipient localities. Restor Ecol 19:433–440

Lebourgeois F, Breda N, Ulrich E et al (2005) Climate-tree-growth relationships of European beech (*Fagus sylvatica L.*) in the French Permanent Plot Network (RENECOFOR). Trees Struct Funct 19:385–401

Leuzinger S, Zotz G, Asshoff R et al (2005) Responses of deciduous forest trees to severe drought in Central Europe. Tree Physiol 25:641–650

Lindner M, Maroschek M, Netherer S et al (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For Ecol Manag 259:698–709

Lof M, Bolte A, Welander NT (2005) Interacting effects of irradiance and water stress on dry weight and biomass partitioning in Fagus sylvatica seedlings. Scand J For Res 20:322–328

Magri D, Vendramin GG, Comps B et al (2006) A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. New Phytol 171:199–221

Magri D (2008) Patterns of post-glacial spread and the extent of glacial refugia of European beech (*Fagus sylvatica*). J Biogeogr 35:450-463

Matyas C, Bozic G, Goemoery D et al (2009) Juvenile growth response of European beech (*Fagus sylvatica L.*) to sudden change of climatic environment in SE European trials. iForest 2:213–220

Messaoud Y, Chen HY (2011) The influence of recent climate change on tree height growth differs with species and spatial environment. PLoS One 6: e14691. doi:10.1371/journal.pone.0014691

Nagy L, Harter D, Backhaus S et al (in prep.) A comparison of genetic and phenotypic variability among beech (*Fagus sylvatica L.*)

provenances from Bulgaria and Germany under drought and temperature manipulation

Nielsen CN, Jorgensen FV (2003) Phenology and diameter increment in seedlings of European beech (*Fagus sylvatica L.*) as affected by different soil water contents: variation between and within provenances. For Ecol Manag 174:233–249

Ohlemuller R, Gritti ES, Sykes MT et al (2006) Quantifying components of risk for European woody species under climate change. Global Change Biol 12:1788–1799

Penuelas J, Hunt JM, Ogaya R et al (2008) Twentieth century changes of tree-ring delta(13)C at the southern range-edge of *Fagus sylvatica*: increasing water-use efficiency does not avoid the growth decline induced by warming at low altitudes. Global Change Biol 14:1076–1088

Petritan AM, Luepke B von, Petritan IC (2007) Effects of shade on growth and mortality of maple (*Acer pseudoplatanus*), ash (*Fraxinus excelsior*) and beech (*Fagus sylvatica*) saplings. Forestry 80:397–412

Peuke AD, Gessler A, Rennenberg H (2006) The effect of drought on C and N stable isotopes in different fractions of leaves, stems and roots of sensitive and tolerant beech ecotypes. Plant Cell Environ 29:823–835

Piovesan G, Biondi F, Di Filippo A et al (2008) Drought-driven growth reduction in old beech (*Fagus sylvatica L.*) forests of the central Apennines, Italy. Global Change Biol 14:1265–1281

Pollastrini M, Desotgiu R, Cascio C et al (2010) Growth and physiological responses to ozone and mild drought stress of tree species with different ecological requirements. Trees Struct Funct24:695–704

Polley, H..; Hennig, P.; Kroiher, F. (2009). Eine Kohlenstoffinventur auf Bundeswaldinventur-Basis: Baumarten, Altersstruktur und Totholz in Deutschland. AFZ-DerWald 64: 1074-1075

R Development Core Team (2010) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, R version 2.11.1. R foundation for statistical computing. ISBN 3-900051-07-0, URL http://www.R-project.org, Vienna, Austria

Rennenberg H, Dannenmann M, Gessler A et al (2009) Nitrogen balance in forest soils: nutritional limitation of plants under climate change stresses. Plant Biol 11:4–23

Rennenberg H, Seiler W, Matyssek R et al (2004) European beech (*Fagus sylvatica L.*) - a forest tree without future in the south of Central Europe? Allg For Jagdztg 175:210–224

Richter S, Kipfer T, Wohlgemuth T et al (2011) Phenotypic plasticity facilitates resistance to climate change in a highly variable environment. Oecologia DOI: 10.1007/s00442-011-2191-x

Rose L, Leuschner C, Koeckemann B et al (2009) Are marginal beech (*Fagus sylvatica L.*) provenances a source for drought tolerant ecotypes? Eur J For Res 128:335–343

Schaberg PG, DeHayes DH, Hawley GJ et al (2008) Anthropogenic alterations of genetic diversity within tree populations: Implications for forest ecosystem resilience. For Ecol Manag 256:855–862

Schall P, Lödige C, Beck M, Ammer C (2012) Biomass allocation to roots and shoots is more sensitive to shade and drought. For Ecol Manag 266:246-253

in European beech than in Norway spruce seedlings

Scharnweber T, Manthey M, Criegee C et al (2011) Drought matters - Declining precipitation influences growth of *Fagus sylvatica L.* and *Quercus robur L.* in north-eastern Germany. For Ecol Manag 262:947–961

Scherrer D, Bader MK, Koerner C (2011) Drought-sensitivity ranking of deciduous tree species based on thermal imaging of forest canopies. Agric For Meteorol 151:1632–1640

Schraml C, Rennenberg H (2000) Sensitivity of different ecotypes of beech trees (*Fagus sylvatica L.*) to drought stress. Forstw Cbl 119:51–61

Shipley B and Meziane D (2002) The balanced-growth hypothesis and the allometry of leaf and root biomass allocation. Funct Ecol 16:326-331

Thomas CD, Cameron A, Green RE et al (2004) Extinction risk from climate change. Nature 427:145–148

Varelides C, Brofas G, Varelides Y (2011) Provenance variation in *Pinus nigra* at three sites in Northern Greece. Ann. Forest Sci. 58:893-900

von Wuehlisch G, Krusche D, Muhs HJ (1995) Variation in temperature sum requirement for flushing of beech provenances. Silvae Genet 44:343–346

Vornam B, Decarli N, Gailing O (2004) Spatial distribution of genetic variation in a natural beech stand (*Fagus sylvatica L.*) based on microsatellite markers. Conserv Genet 5:561–570

Wang T, Hamann A, Spittlehouse DL et al (2006) Development of scale-free climate data for Western Canada for use in resource management. Int J Climat 26:383-397

Weitner A, Dupouey JL, Lefevre Y et al (2007) Roles of soil chemistry and water availability in site-related delta C-13 variations in French beech forests. Tree Physiol 27:1043–1051

Wortemann R, Herbette S, Barigah TS et al (2011) Genotypic variability and phenotypic plasticity of cavitation resistance in *Fagus sylvatica L.* across Europe. Tree Physiol 31:1175–1182

6.4. Manuskript 4:

Ecotypes of European grass species respond differently to warming and extreme drought

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SPECIAL FEATURE: ECOLOGICAL CONSEQUENCES OF CLIMATE EXTREMES

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Key-words

adaptation, climate change, climatic events, extreme weather, grassland, meadows, phenotypes, plant–climate interactions, provenances, within-species variability

Summary

1. Extreme climatic events are expected to increase in frequency and magnitude as a consequence of global warming.

2. Managed permanent grasslands cover a large surface in Europe and contribute substantially to agricultural production. These managed plant communities are dominated by perennial clonal species. Their capacity to adapt to rapidly changing environmental conditions may be limited.

3. We hypothesize that those plant populations that have already been exposed to conditions that are expected to occur due to future climate change, particularly conditions that would be ‘extreme’ in the target area, are able to cope better with these conditions.

4. For a common-garden experiment we selected ecotypes (provenances as supported by accessions in seed banks) of important European grass species: *Arrhenatherum elatius*, *Festuca pratensis*, *Holcus lanatus* and *Alopecurus pratensis*. Southern target locations of

ecotypes (populations) were identified based on climate model projections for the local site in Northern Bavaria, Germany.

5. In a controlled experiment, the plants were exposed to warming and extreme drought. Drought conditions(16-19 days, depending on the species) were imposed starting from the end of May in combination with and without an increase in the average temperature from May to September 2009 (+1.5 K compared to control; +2.5 K compared to ambient conditions outside of the experimental units).

6. Ecotypes and drought manipulation had significant impacts on biomass production and tissue die-back. Significant interactions between ecotype and drought indicated a different drought tolerance of the ecotypes in some cases. The warming treatment yielded a less significant response. The local ecotype generally did not perform significantly worse than the presumably better-adapted southern ecotypes.

7. *Synthesis.* The selection of ecotypes that are adapted to more extreme climatic conditions could be an option for maintaining future ecosystem functioning in temperate managed grasslands, as was indicated by the clear differences between ecotypes in our experiment. Based on our data, however, performance cannot be predicted from climatic origin. Therefore, we recommend enhancing the genetic variability within populations of species in general.

Introduction

One of the consequences anticipated from global warming and the subsequently stimulated energy exchange in the atmosphere is an increase in the frequency and magnitude of extreme climatic conditions (Meehl *et al.* 2000; Beniston & Stephenson 2004; IPCC 2007; Sillmann & Roeckner 2008; O'Gormann & Schneider 2009). These extreme events are expected to have above-average impacts on plants and ecosystems and could contribute to the surpassing of ecosystem thresholds (Gutschick & BassiriRad 2003; Reusch *et al.* 2005; Jentsch 2006; Jentsch & Beierkuhnlein 2008; Knapp *et al.* 2008; Jentsch *et al.* 2011; Smith 2011). The approach of many ecological projections is to link long-term trends in average climatic conditions (e.g. 30-year mean precipitation) to the average behaviour or site selection of organisms as a result of established biotic interactions. This does not take into account the increasing temporal variability in climatic regimes.

The current understanding of atmospheric global warming processes results in the expectation of increased occurrence and risks of extreme weather events (Smith *et al.* 2009). Heat waves,

floods, droughts and wildfires in particular are expected to become even more frequent and intense than was expected some years ago (for summer drought in Europe see Rowell 2008), with alarming consequences for terrestrial ecosystems.

Responses of ecosystems to climatic changes can be found in various biomes of the earth, ranging from species to communities (Walther *et al.* 2002). Effects are modified by vegetation structures, plant species composition and diversity, and the key plant species that dominate the functioning of communities. Experimental approaches to simulate the impacts of extremes in the precipitation regime (periods of drought or heavy rain) and of warming have been established in various ecosystems (Beier *et al.* 2004; Jentsch, Kreyling & Beierkuhnlein 2007; Fay *et al.* 2008; Smith 2011).

It is most likely that warming will increase biomass production in various ecosystems (Rustad *et al.* 2001). However, projections of ecosystem performance are difficult mainly because of uncertainties in the future distribution of water. The projected speed of climate change can exceed the dispersal capacities of many plant species. Even if long-term adjustments can be expected, on the time scale of decades and perhaps even centuries, regional losses of biodiversity are likely to occur. Furthermore, rapid shifts towards novel site conditions at a given site may not be followed by the organisms. Extreme events can cause the local breakdown of populations (Breshears *et al.* 2005). This could result in declining ecosystem functionality. Consequences for ecosystem services are therefore to be expected.

Hay meadows are of high importance for European agriculture in temperate and humid regions and occupy a large proportion of the landscapes (13.2% of the total area or 568 042 km^2 within the EU 27) (EUROSTAT 2010). Permanent grasslands are defined in the EU as agricultural surfaces that are under land use for several, usually more than five, consecutive years and are covered by herbaceous forage crops, either naturally (through self-seeding) or through cultivation (sowing). They are not included in the crop rotation scheme on the agricultural holding (EUROSTAT 2010) and can be either managed as pastures with grazing livestock or through mowing for hay or silage. Such grasslands are dominated usually by only a few key species. In order to evaluate the future performance of grassland ecosystems, it is crucial to understand thresholds for the population survival of these species. In contrast to managed grasslands that are annually ploughed and sown (e.g. with *Lolium perenne*), permanent grasslands are rich in species and contribute largely to regional biodiversity.

In spite of being subject to anthropogenic land use over a long time period, key European grass species in permanent grasslands display large-scale genetic patterns (Michalski *et al.*

2010). In extensively used hay meadows of Central Europe, the selected species for this experiment—*Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl*, Festuca pratensis* Huds. s.l., *Holcus lanatus* L*,* and *Alopecurus pratensis* L.—play a major role. These species were part of the German BIODEPTH experiment (Hector *et al.* 1999), which is located nearby the EVENT-Experiment.

Individuals of local and regional populations develop a pool of phenotypes that can be assumed to be best adapted to their specific environment if time is sufficient and the environment is stable. In fact, only an incomplete spectrum of phenotypes of a taxon can be represented in a local pool, and genetic diversity within the population is limited by dispersal history or filters. Only a few studies have considered genotypes or phenotypic responses in grass species experimentally (e.g. Fetcher & Shaver 1990; Ryser & Aeschlimann 1999). In fact, the role of within-species genetic and functional diversity for the response of a species to climate change has been widely neglected in recent research. Especially for widespread species, spatial and genetic distances between populations have to be taken into account.

The objective of this study was to explore mechanisms critical for understanding the preservation and adaptation of ecosystems in the face of climate change. We wanted to identify whether ecotypes (represented in local populations) of certain key species from different locations (provenances) also differ in their response to extreme climatic conditions. If this were found, species populations or ecotypes could be sought that are better adapted to the expected climate of the future than regional populations. Here, we focus on plant traits related to the key ecosystem function productivity: biomass and necrotic tissue.

Material and Methods

Experimental Site

This common-garden experiment (EVENT 3) is part of the EVENT-experiments (Jentsch, Kreyling & Beierkuhnlein 2007; Jentsch & Beierkuhnlein 2010) and was established in March 2009. It is located in Bayreuth, Germany, on the property of the Ecological–Botanical Gardens of the University of Bayreuth in direct proximity to EVENT 1 and EVENT 2 (49°55’19” N, 11°34’55” E). The long-term mean annual temperature for the site is 8.2 °C, whereas the long-term mean annual precipitation is 724 mm. Precipitation is distributed bi-modally with a major peak in June/July and a second peak in December/January (data: German Weather Service).

Under the A1B scenario (IPCC 2007) regional climate models (REMO, BfG 2009) have projected the following climatic conditions for the site. Annual average temperature (30-year mean) is expected to increase by 2.4 K by the middle of the century (2041/2070 compared to 1971/2000) and by 3.5 K by the end of the century (2071/2100 compared to 1971/2000). Precipitation is not expected to change considerably according to the annual mean values (+ 2 mm $year^{-1}$ 2071/2100), but summers are expected to become dryer (-17 mm) over the same period.

Selection of Species and Ecotypes

The species that were selected for this study are common in Central European managed grasslands. *Arrhenatherum elatius* is a tallgrass that contributes substantially to biomass production in meadows. It is a widespread and common species in Europe, with a high abundance in permanent temperate grassland. It is found on moist to moderately dry nutrient-rich soils (Oberdorfer 2001). Due to its importance as a forage plant, this phenotypically variable grass was cultivated and planted outside of its natural range. *Festuca pratensis,* with its wide distribution across Europe, is a high-quality forage plant that is also suitable for pastures. It grows predominantly on fresh soils rich in nutrients and humus and has been promoted through cultivation (Oberdorfer 2001). *Holcus lanatus* occurs in all European countries, predominantly on wet and boggy soils (Oberdorfer 2001), where it is of a certain importance as a forage plant; it is less common on pastures compared to meadows. *Alopecurus pratensis* is a common grass found throughout most of Europe in moist and nutrient-rich soils (although also less common on pastures) that produces abundant and high-quality forage (Oberdorfer 2001). For this reason, it is also planted and cultivated outside of its natural range. As perennial clonal grasses, all four species share comparable life cycles and strategies for resource allocation.

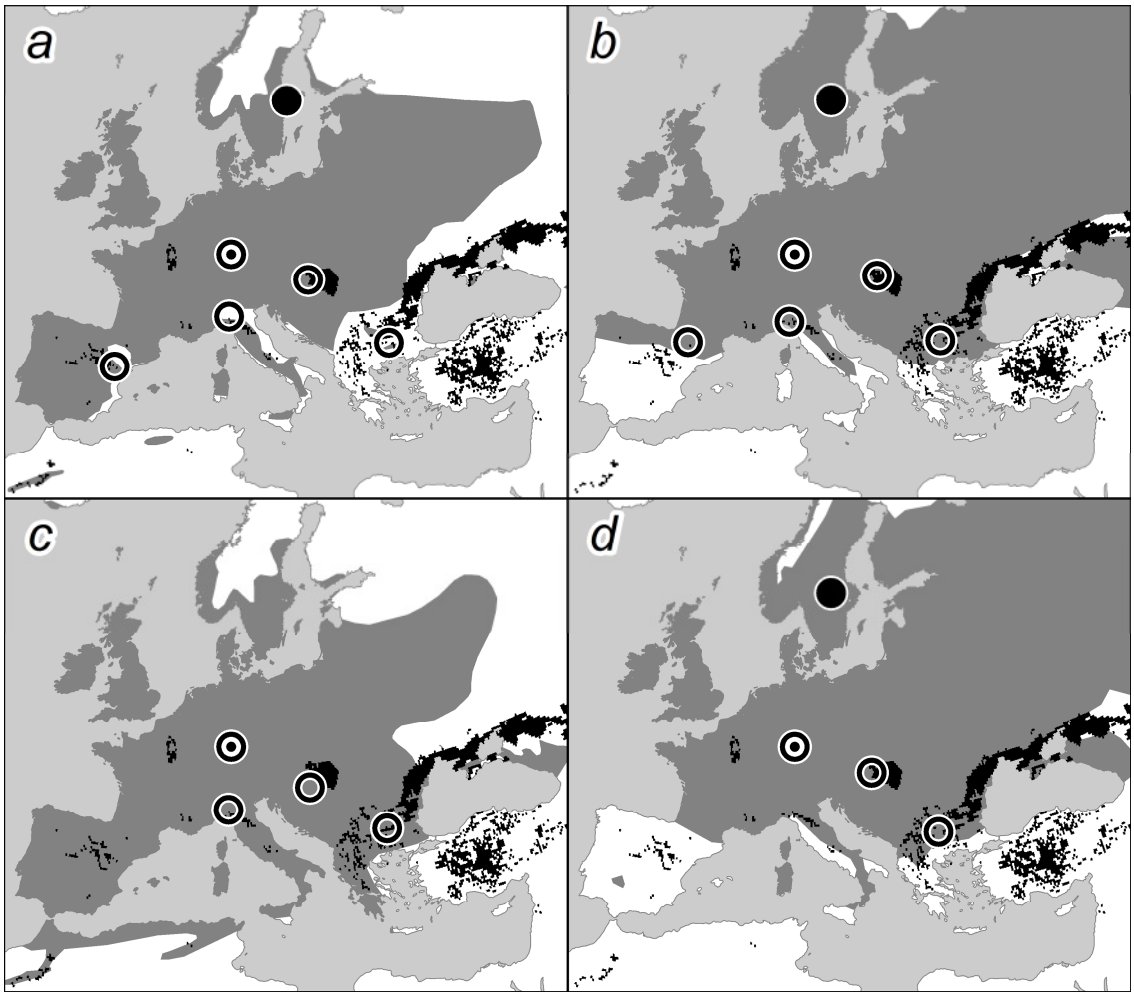

Figure 1: Selected ecotypes of (3a) *Arrhenatherum elatius*, (3b) *Festuca pratensis,* (3c) *Holcus lanatus* and (3d) *Alopecurus pratensis*. The shaded area indicates the species distribution. Black areas mark the regions with current climatic conditions similar to the projected future climate of the German location. The local ecotype is displayed as target (DE Germany). The filled circle shows location of the ecotype from northern reference populations (SE Sweden). The empty circles indicate southern ecotypes from regions that are climatic analogues to future expectations for the local climate (ES Spain, IT Italy, HU Hungary, BG Bulgaria).

Besides local ecotypes of these four species from Germany (DE), we selected other European ecotypes of these grasses on the basis that the climate of the region of origin was similar to the local projections for our site in northern Bavaria in the future. Local projections of mean annual temperature, mean winter temperature and annual precipitation for the period 2071-2100 were taken from the regional climate model REMO (BfG 2009) based on the A1B Scenario (IPCC 2007). Regions with current climatic conditions similar to the future projections for the target area were located based on worldclim data (Hijmans *et al.*, 2005). Seed material was obtained from these target regions (Fig. 1), abbreviated below as Spain (ES), Italy (IT), Hungary (HU) and Bulgaria (BG). Furthermore, in order to test whether northern populations differ, we added a region from the northern part of the species' ranges (in our case Sweden, SE). For *A. elatius* and *F. pratensis* ecotypes were available from seed

banks for all target regions. For *H. lanatus* and *A. pratensis* only four ecotypes were available (Table 1).

Table 2: Site information (DE Germany, SE Sweden, ES Spain, IT Italy, HU Hungary, BG Bulgaria) for the accessions of ecotypes (provenances) of *Arrhenaterum elatius*, *Festuca pratensis*, *Holcus lanatus* and *Alopecurus pratensis* used in the EVENT 3 experiment. Temperature (T) and precipitation (P) represent long-term mean annual values (source: WORLDCLIM). Accession responds to the code of the seed bank at IPK Poel (codes beginning with GR from IPK seed bank, codes with PI from USDA-ARS-GRIN, codes with NordGen, Bf and RCAT from Eurisco)

		Location	Accession	Latitude	Longitude	Elevation (m a.s.l.)	T (°C)	P (mm)
A. elatius	DE	Blaufelden, Hohenloher Ebene	GR 12752	49°17'46"N	09°58'25"E	460	8.3	732
	SE	Uppsala	PI 235543	59°51'25"N	17°38'22"E	20	5.7	551
	ES	Montalban	PI 234465	40°50'07"N	00°47'55"E	900	11.0	450
	IT	Rizzolo, Emilia-Romagna	GR 12733	44°55'12"N	09°44'46"E	110	12.9	739
	HU	Lókút	RCAT064783	47°12'00"N	17°52'00"E	440	8.9	621
	BG	NA	BGR: 2008-ARR-EL-1	42°00'00"N	24°50'00"E	NA	NA	NA
F. pratensis	DE	Blaufelden, Hohenloher Ebene	GR 12753	49°17'46"N	09°58'25"E	460	8.3	732
	SE	Mårtenstorpet, Grängesberg	NordGen: 1191	60°00'00"N	15°00'00"E	350	4.3	738
	ES	San Anton	Bf 1592	42°37'08"N	00°09'47"W	1250	6.8	989
	IT	Lago Nero, Ferriere Piacenza	Bf 1078	44°33'00"N	09°27'00"E	1600	8.5	981
	HU	Vérteskozma (Gánt)	RCAT040707	47°27'00"N	18°28'00"E	270	10.2	571
	BG	Dolna Banya	GR 6976/99	42°19'05"N	23°45'07"E	710	9.6	585
H. lanatus	DE	Blaufelden, Hohenloher Ebene	GR 12750	49°17'46"N	09°58'25"E	460	8.3	732
	IT	Torrano, Emilia-Romagna	GRA 312	44°53'33"N	09°41'20"E	160	12.6	758
	HU	Horváthertelend	RCAT040972	46°10'00"N	17°55'00"E	200	10.9	675
	BG	Mihiltsi	GR 6632/00	42°31'19"N	24°48'52"E	330	10.9	581
A. pratensis	DE	Blaufelden, Hohenloher Ebene	GR 12751	49°17'46"N	09°58'25"E	460	8.3	732
	SE	Mårtenstorpet, Grängesberg	NordGen: 1183	60°00'00"N	15°00'00"E	350	4.3	738
	HU	Lókút	RCAT064581	47°12'00"N	17°52'00"E	440	8.9	621
	BG	Iskar Dam	GR 6635/00	42°26'29"N	23°35'20"E	810	9.0	593

The Cultivation of the Plants

The target ecotypes were cultivated at the branch office of the *Leibniz Institute of Plant Genetics and Crop Plant Research* (IPK) in Poel, Germany, from February 2009 to April 2009. The seedlings were then transported to Bayreuth and individually planted into 4-L

plastic pots filled with a local forest-topsoil. The soil substrate was sandy silt (pH 7.27, total C 1.89%, total N 0.15%, plant-available $NH4^+$ 1.79 mg L^{-1}, plant-available $NO3^-$ 22.50 mg L^{-1}). For the first two weeks after planting, the seedlings were watered generously with tap water to ensure growth. All individuals were then cut to a height of 7 cm in order to create comparable starting conditions and exposed to ambient precipitation until the start of the experimental treatments on 25 May 2009. The experiment ended in September 2009.

Experimental Design

The potted individuals of the selected ecotypes were planted in pots and exposed to temperature treatments (warming and control) and to precipitation treatments (extreme summer drought and control) in a split-plot design. The two climate treatments were fully crossed, resulting in four climate manipulations (control, drought, warming, warming & drought), which were replicated three times, resulting in 12 experimental units in total. The ecotype treatment was nested within each experimental unit. Each ecotype was further replicated with seven plants per experimental unit (nested replicates). The available plants were assigned randomly to the 12 experimental units for each species. Each experimental unit was covered by a single rain-out shelter constructed of a steel frame (GlasMetall Riemer GmbH, Rahden- Sielhorst, Germany) and covered with a transparent polyethylene sheet (0.2 mm, SPR5, Hermann Meyer KG, Rellingen, Germany). The edge of the rain-out shelters was at a height of 80 cm and permitted nearly 90% penetration of photosynthetically active radiation.

The control precipitation regime simulated the local daily 30-year average precipitation. The application was done twice a week with collected rain water.

The extreme drought treatment consisted of a period without precipitation. The definition of duration is based here on the species-specific response of organisms: A four-stage key (0 to 3, where 0 stands for ‘completely undamaged’ and 3 stands for ‘totally dried out and brittle’) was developed to describe the amount of visible damage caused by the drought. By the time two-thirds of the individuals of one species had reached stages 2 or 3, or by the time one third of the individuals had reached stage 3, the drought was stopped. The drought treatment lasted 16 days for *H. lanatus*, 18 days for *A. pratensis* and *F. pratensis* and 19 days for *A. elatius*. The drought treatment resulted in a dropping of soil moisture below the permanent wilting point of the soil approximately one week after the start of the treatment (Fig. 2).

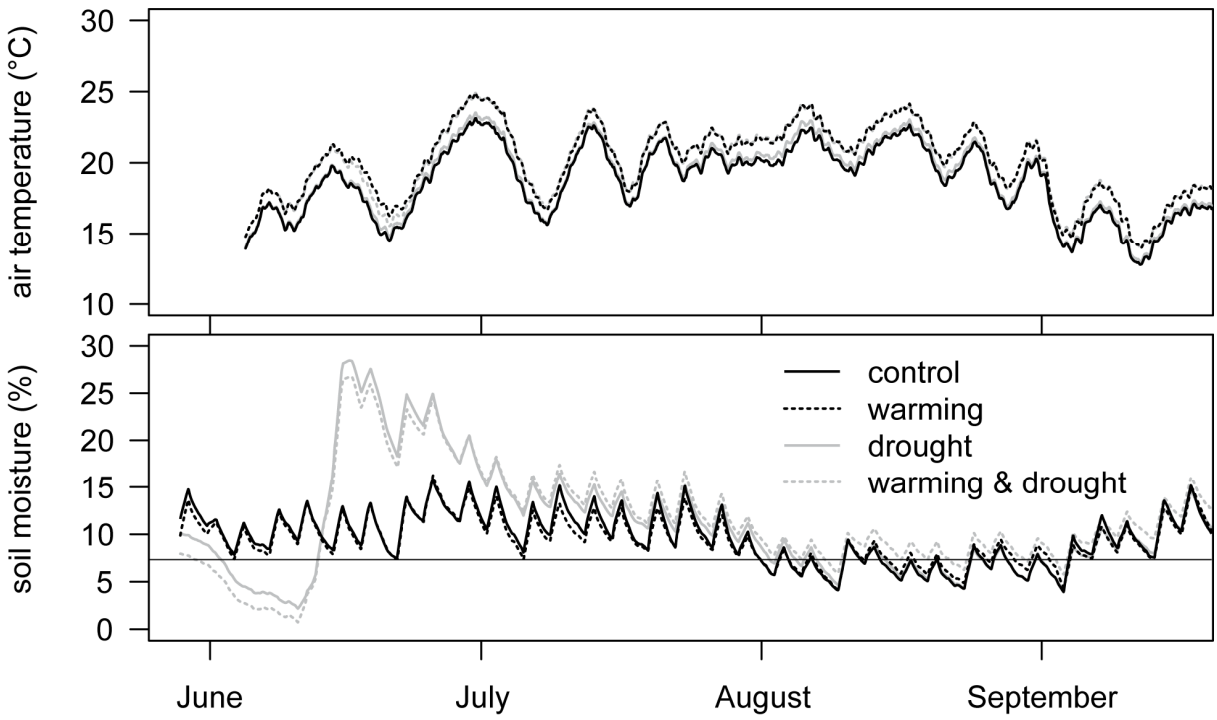

Figure 2: Air temperature at plant height and soil moisture (-2 to -7 cm) during the experiment. Air temperature was measured in 10-min intervals at two locations within each experimental unit by sheltered thermistores (B57863-S302-F40, EPCOS) connected to a datalogger (dl2, Delta). Soil moisture was measured hourly by FD-sensors (Echo.EC-5/k, Decagon Devices, Pullmann (WA), USA) at one randomly assigned pot for each species within each experimental unit (n = 12 per treatment). Mean values over all species are shown as no detectable difference between species occurred. The horizontal dotted line represents the approximate permanent wilting point (pF = 4.2).

The warming treatment was performed continuously throughout the whole experiment. This was done passively via wind-shelters and black floor-covers, which increased the average temperature by 1.5 K compared to the temperature control treatment and by 2.5 K compared to the ambient temperature outside of the experimental units (Fig. 2).

The fourth treatment was a combination of extreme drought and warming. The additional warming increased the drought treatment effect by additionally reducing the soil moisture by about 1.5% on average (Fig. 2). In the re-watering phase each individual in the drought and combined treatments received 350 mL on three consecutive days (1050 mL in total), which corresponds to 38 mm of precipitation. This re-watering resulted in a steep increase of soil moisture (up to 28%). Afterwards, the pots were watered according to the control precipitation treatment. Soil moisture after re-watering remained higher in the drought-manipulated pots than in the control pots for nearly one month. Soil moisture fell repeatedly below the permanent wilting point for short periods in August due to unusually high ambient temperatures.

Biomass

Based on local agricultural management routines of extensive grasslands, biomass was harvested twice over the growing season. The first biomass harvest took place ten days after the drought treatment ended, respectively, for each species, in order to account for the recovery capacity of the plants. Each individual plant was cut at 3 cm above the soil and the biomass was dried for 48 h at 70 °C and weighed. For three out of the seven nested replicates per experimental unit, the biomass was divided into living and necrotic material and then processed as mentioned.

The second harvest was conducted 72 days after the first harvest on 3 September 2009 with the same procedure, except that the harvested material was not split into living and necrotic biomass.

Statistics

Linear mixed-effect models were applied for each species separately to test for the main and interactive effects of the three factors: ecotype, temperature treatment and precipitation treatment. The split-plot design and the nested replicates were accounted for by the use of the experimental unit identity as a random factor (Pinheiro & Bates 2004). Data were log-transformed to improve the homogeneity of variances and the normality of residuals prior to analysis, if necessary (Faraway 2006). In case of significant ecotype or interaction effects, *post hoc* comparisons (Tukey's test) were run according to Hothorn, Bretz & Westfall (2008). All statistical analyses were conducted with the software R 2.11.1 and the additional packages 'nlme', 'multcomp' and 'sciplot'.

Results

Biomass

Biomass production differed significantly between the ecotypes for all four species and both harvests (Table 2). No single ecotype performed superior to the others when viewed over all four species, and the local ecotype (DE) was significantly outperformed by a southern ecotype in only one out of eight cases (*H. lanatus* at the first harvest; Fig. 3a). Interestingly, variability in biomass production within species was comparable to variability between species (Table

3). This pattern was independent from drought and warming manipulations as it did not differ when data were averaged over the whole data set or analysed per single treatments. At the first harvest, *A. elatius* showed highest variability between ecotypes, which even exceeded variability between species. The same was true for *H. lanatus* for the second harvest.

The drought treatment resulted in a significant reduction of biomass production for all four species in the first harvest, while only *F. pratensis* and *H. lanatus* were still negatively impacted by the drought in the second harvest (Table 2). Warming did not alter biomass production significantly except for a slight increase in biomass production for *F. pratensis* in the first harvest (Table 2).

In the first harvest, ecotypes only differed significantly in their drought tolerance for *A. elatius* (interaction between ecotype and drought: $p = 0.005$). Here, the German, Italian, Hungarian and Bulgarian ecotypes showed the best performance with similar reductions in biomass production due to drought, while the Swedish ecotype exhibited higher drought sensitivity and the Spanish ecotype generally produced less biomass (Fig. 3).

Differences in sensitivity to drought became more apparent over time, with *A. elatius*, *H. lanatus* and *A. pratensis* showing significant interaction effects between ecotype and drought at the second harvest (Table 2). For *A. elatius*, the ecotypes with a better performance from the first harvest tended to increase biomass production in the drought manipulation compared to the control (DE, IT, HU, BG), while the other two ecotypes (ES and SE) showed no similar trend of compensatory growth (Fig. 3).

The Italian ecotype of *H. lanatus* performed best at the first harvest (Fig. 3a) and—together with the German ecotype—also at the second harvest (Fig. 3b). No interaction was found between ecotype and the climate treatments for the first harvest, while the reaction to drought differed between ecotypes at the second harvest (Table 2). Here, biomass production by the Italian and the German ecotypes was not significantly affected when comparing control and drought, while a reduction in biomass due to the drought treatment was evident in the Hungarian and Bulgarian ecotypes (Fig. 3).

Alopecurus pratensis exhibited the hypothesized pattern of better performance among the southern ecotypes (HU & BG) and worse performance than the northern ecotype (SE), with the German ecotype intermediate and not significantly different from any of the three others for the first harvest (Fig. 3a). The Bulgarian ecotype, however, fell behind in terms of biomass compared to the Hungarian and the German one, while the Swedish ecotype no longer differed significantly from the others at the second harvest. It was only for the second harvest

that a significant interaction between ecotype and drought was evident (Table 2) with the German ecotype being most productive in both the drought and the control treatments, while the Bulgarian ecotype produced less biomass in the control treatment and the Swedish ecotype produced less biomass in the drought treatment.

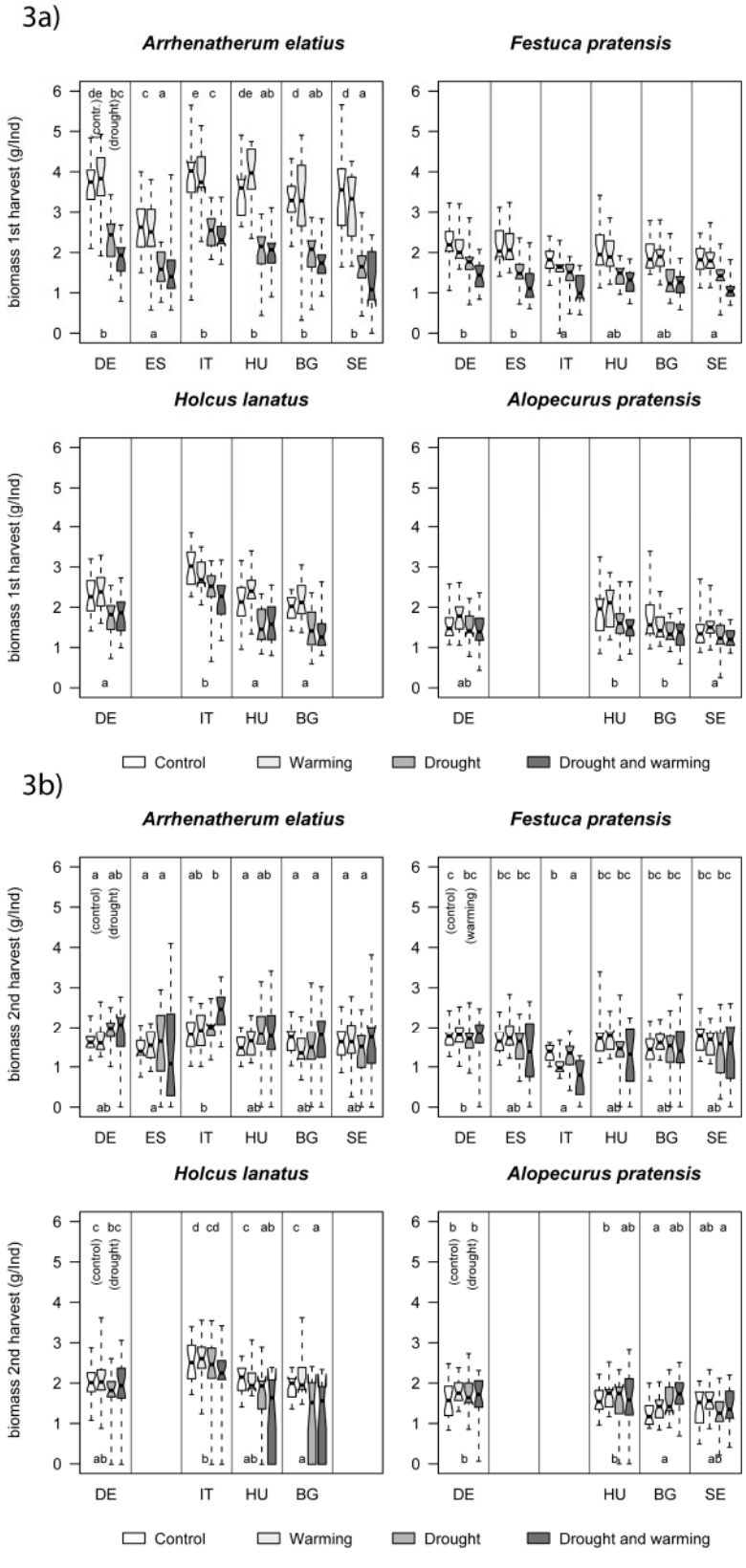

Figure 3: Effects of climate treatments and ecotypes (DE Germany, SE Sweden, ES Spain, IT Italy, HU Hungary, BG Bulgaria) on biomass production in g per individual of the four grass species. Lower-case letters below the boxplots show homogeneous groups according to post-hoc comparisons in case of significant main effects for the factor ecotype (see Table 2 for ANOVA results). Lower-case letters above the boxplots indicate homogeneous groups according to post-hoc comparisons in case of significant interaction effects between ecotype and the specified climate treatment. The upper and lower edges of the boxes represent the 25%- and the 75%-quantile, the black line within the boxes the median (50%-quantile) and the upper and lower whiskers the whole variance in biomass production. 3a) First harvest (June). 3b) Second harvest (early September).

Table 3: ANOVA results of the applied mixed models. Analyses were run for each species and response parameter separately. Displayed are the degrees of freedom (d.f.), F- and p-statistics. See Materials and methods for details on model specifications.

		Arrhenaterum elatius				*Festuca pratensis*				*Holcus lanatus*				*Alopecurus pratensis*			
		num d.f.	Den d.f.	F	p	num d.f.	Den d.f.	F	p	num d.f.	Den d.f.	F	p	num d.f.	Den d.f.	F	p
1st harvest	ecotype	5	470	25.5	< 0.001	5	472	13.1	< 0.001	3	312	44.5	< 0.001	3	311	12.1	< 0.001
	drought	1	8	187.0	< 0.001	1	8	83.8	< 0.001	1	8	55.9	< 0.001	1	8	8.8	0.018
	warming	1	8	0.6	0.466	1	8	7.9	0.023	1	8	0.1	0.709	1	8	0.0	0.981
	ecotype×drought	5	470	3.4	0.005	5	472	1.9	0.100	3	312	0.4	0.724	3	311	0.9	0.448
	Ecotype×warming	5	470	1.6	0.169	5	472	0.7	0.635	3	312	1.9	0.125	3	311	1.2	0.318
	drought×warming	1	8	1.0	0.342	1	8	1.4	0.266	1	8	0.4	0.570	1	8	0.1	0.739
	ecotype×drought×warming	5	470	0.6	0.707	5	472	0.3	0.903	3	312	1.0	0.412	3	311	1.2	0.301
2nd harvest	ecotype	5	470	8.3	< 0.001	5	472	15.3	< 0.001	3	312	20.6	< 0.001	3	311	5.9	< 0.001
	drought	1	8	0.9	0.366	1	8	7.4	0.027	1	8	20.0	0.002	1	8	0.0	0.953
	warming	1	8	0.3	0.600	1	8	1.1	0.317	1	8	0.0	0.889	1	8	0.3	0.623
	ecotype×drought	5	470	2.3	0.046	5	472	1.3	0.258	3	312	3.6	0.014	3	311	4.4	0.005
	ecotype×warming	5	470	0.9	0.508	5	472	2.9	0.015	3	312	1.2	0.313	3	311	0.6	0.632
	drought×warming	1	8	0.1	0.793	1	8	0.9	0.375	1	8	0.5	0.488	1	8	0.5	0.501
	ecotype×drought×warming	5	470	1.2	0.330	5	472	0.4	0.852	3	312	0.2	0.873	3	311	0.9	0.433
necrotic tissue	ecotype	5	183	13.5	< 0.001	5	183	4.1	0.002	3	120	11.8	< 0.001	3	120	0.1	0.969
	drought	1	8	172.1	< 0.001	1	8	40.4	< 0.001	1	8	160.4	< 0.001	1	8	74.7	< 0.001
	warming	1	8	0.1	0.717	1	8	2.4	0.164	1	8	0.4	0.547	1	8	0.2	0.665
	ecotype×drought	5	183	2.1	0.064	5	183	12.8	< 0.001	3	120	1.1	0.358	3	120	4.5	0.005
	ecotype×warming	5	183	1.8	0.117	5	183	0.9	0.481	3	120	0.5	0.708	3	120	0.3	0.841
	drought×warming	1	8	1.8	0.216	1	8	0.8	0.386	1	8	0.6	0.457	1	8	0.7	0.423
	ecotype×drought×warming	5	183	0.1	0.996	5	183	0.8	0.585	3	120	0.5	0.663	3	120	0.9	0.447

Only one species showed an ecotype-specific reaction to the warming treatment (*F. pratensis*, 2nd harvest, Table 2). This effect was due to a significant reduction in biomass for the warmed versus the control treatment for the Italian ecotype (Fig. 3), with no other ecotype showing such a reaction to the treatment.

Die-Back

Necrotic tissue occurred mainly in the drought treatment (Fig. 4). The variation in the amount of necrotic leaves between the single plants across all species was much higher in the drought treatments (drought: up to 100% necrotic tissue, drought and warming: up to 100%) compared to control (up to 31% necrotic tissue, two outliers not included) and warming-only treatments (up to 40%). Nevertheless, the proportion of necrotic tissue varied significantly between the ecotypes in all species except *A. pratensis* (Table 2). The local ecotype again did not perform significantly worse than the southern ecotypes for any of the species (Fig. 4). On the contrary, it turned out to be one of the best-performing ecotypes. Warming had no single or interactive effect on the proportion of necrotic tissue for any of the four species, which indicates that higher temperatures did not increase water stress.

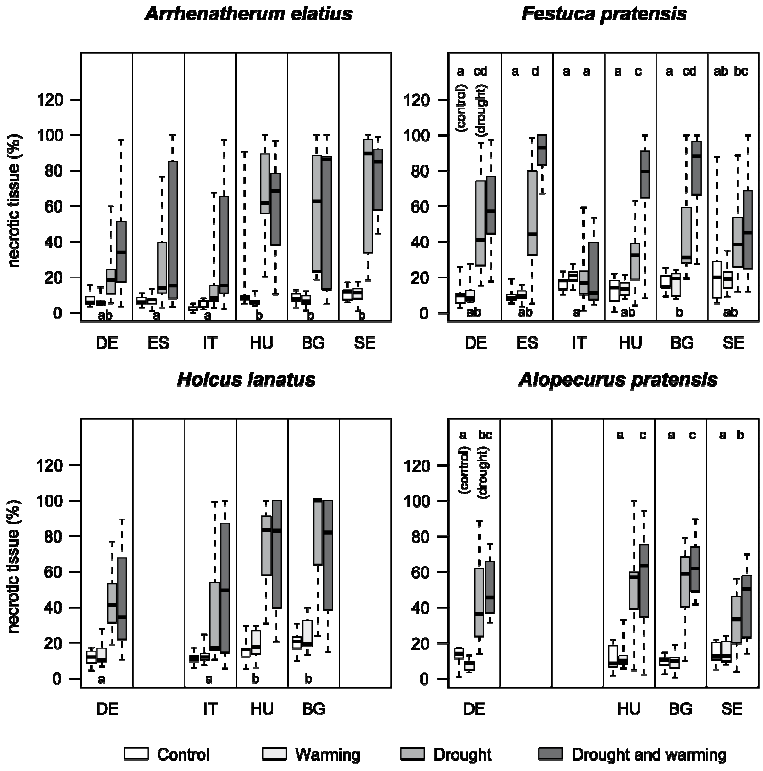

Figure 4: Effects of climate treatment and ecotype (DE Germany, SE Sweden, ES Spain, IT Italy, HU Hungary, BG Bulgaria) on the proportion of necrotic plant material in the first harvest (June). Values are in % of the total biomass production per individual of the four species. Lower-case letters below the boxplots show homogeneous groups according to post-hoc comparisons in case of significant main effects for the factor ecotype (see Table 2 for ANOVA results). Lower-case letters above the boxplots indicate homogeneous groups according to post-hoc comparisons in case of significant interaction effects between provenance and the specified climate treatment. The upper and lower edges of the boxes represent the 25%- and the 75%-quantile, the black line within the boxes the median (50%-quantile) and the upper and lower whiskers the whole variance percentage of necrotic tissue.

For *F. pratensis*, the Italian ecotype showed the best performance in terms of resistance against tissue die-back. Only this ecotype and the Swedish one did not exhibit a significant increase in the drought treatment compared to the control (Fig. 4). The Swedish ecotype also showed the least response to drought for *A. pratensis*, which was the other species with a significant interaction between ecotype and drought treatment (Table 2).

Table 4: Comparison of mean values (g dry weight per pot) and standard deviations (sd) of biomass production within and between species and climate manipulations.

harvest	climate manipulation	*Arrhenatherum elatius*		*Alopecurus pratensis*		*Festuca pratensis*		*Holcus lanatus*		*total sd*
		mean	sd	mean	sd	mean	sd	mean	sd	
first	control	3.4	0.89	1.7	0.58	2.0	0.48	2.4	0.61	***0.95***
	drought	2.0	0.66	1.4	0.40	1.5	0.40	1.8	0.66	***0.60***
	warming	3.4	0.95	1.7	0.46	1.9	0.45	2.5	0.47	***0.95***
	warming & drought	1.8	0.67	1.4	0.39	1.2	0.37	1.7	0.58	***0.58***
	total	*2.7*	***1.10***	*1.6*	***0.48***	*1.7*	***0.54***	*2.1*	***0.67***	***0.89***
second	control	1.6	0.38	1.5	0.43	1.6	0.40	2.1	0.48	***0.48***
	drought	1.7	0.71	1.5	0.49	1.4	0.56	1.7	0.94	***0.69***
	warming	1.6	0.45	1.6	0.36	1.6	0.41	2.3	0.51	***0.51***
	warming & drought	1.8	0.96	1.5	0.64	1.2	0.79	1.6	0.96	***0.87***
	total	*1.7*	***0.66***	*1.5*	***0.49***	*1.5*	***0.58***	*1.9*	***0.80***	***0.66***

Discussion

Ecotype Effects

In Europe, the native grass species included in this study (*Arrhenatherium elatius*, *Festuca pratensis*, *Holcus lanatus*, *Alopecurus pratensis*) have a large distribution area and grow under highly contrasting conditions (see also Humphreys *et al.* 2006). Perennial species that cover such a broad range of climatic conditions can be expected to exhibit climatically adapted ecotypes (Macel *et al.* 2007; Ofir & Kigel 2010).

In our study, the geographic origin significantly influenced biomass production on both harvest dates for each of the four grass species. For necrotic tissue such geographical influences were observed for three of the species, but not for *A. pratensis* (Table 2).

The local ecotype did not generally perform significantly worse than the respective best performing ecotype. Macel *et al.* (2007) found comparable 'home site advantages' for *H. lanatus.* In our experiment, all pots were kept under roofs, which enabled us to control the water supply. Even though we simulated warming and drought, the environmental conditions did not differ dramatically from local site conditions. It is likely that the treatments applied did not exceed the adaptation capacity of local populations.

Generally, we found that variability in the biomass production between species did not exceed variability within species. In some cases, intra-specific variation even exceeded inter-specific variation by more than 20% (Table 3). This remarkable finding indicates that the variability of ecotypes within a species can hardly be ignored, especially when projections on the responses to climatic changes are made or when dispersal is modelled.

Drought and Warming Effects

In this experiment, all grass species responded significantly to drought. This is not surprising as the duration of the manipulation was based on the plant response. However, the applied drought periods of 16 to 19 days are well within realistic scenarios for the future and even for the past on our site (Jentsch, Kreyling & Beierkuhnlein 2007). Furthermore, the soil moisture conditions and the time span below the wilting point in our experiment (Fig. 2) closely resemble the soil moisture conditions in nearby field experiments during the simulation of local drought events with a 1000-year recurrence based on the local climate data series (Jentsch *et al*. 2011).

In Central Europe, summer precipitation is expected to decrease (BfN 2009). In addition, climate change is predicted to be accompanied by increased climatic variability, and therefore there will be a higher probability of drought periods. Our results indicate that European managed grasslands, which are dominated by the grass species used in this study, might be prone to strong fluctuations in biomass production, as reported for the drought of 2003 (Ciais *et al.* 2005). These effects might compensate the positive effects of an extended growing season in a milder climate.

In artificial plant communities at the same site as this experiment, Jentsch *et al.* (2009) identified species-specific responses to drought and increased precipitation, which depended on species diversity and competition. The effects of community type and composition were also shown for biomass production and tissue die-back of single species following experimental climatic extremes (Kreyling *et al.* 2008). Furthermore, Mirzaei *et al.* (2008) found that temporally limited but extreme drought events may cause strong repercussions in the carbon cycling of grassland communities. This result indicates that species responses should be regarded in the context of communities (Jentsch *et al.* 2011). The advantage of common-garden experiments is that they can detect the spectrum of species-specific responses, which seems to be imperative when these species are applied in seed mixtures for the establishment and management of grasslands. Long-term monitoring of drought effects in grasslands proved that productivity reacted directly to summer drought (Morecroft *et al.* 2004).

Surprisingly, additional warming in our experiment did not aggravate the reactions, indicating that water shortage is the major driver of declining performance. The moderate effect of warming could be due to the fact that we only applied a slight increase in temperature (+ 1.5 K compared to the control and + 2.5 K compared to ambient conditions outside of the experimental units) for a short duration (May to September). The warming was not intended to be extreme, but to simulate the expected average background temperature? in the near future (middle of the 21st century). Thus, we can neither detect nor generalize that warming would not be relevant. Extreme heat waves are expected to occur that could have comparable impacts to the extreme drought conditions applied in this experiment (Clark, Murphy & Brown 2010).

In addition, perennial grass species do not necessarily reach optimum growth at the same age. Thus, our results on juvenile plants cannot be extrapolated as a general pattern of response to drought for the respective species. Moreover, we did not analyse the species-specific spatial

patterns of below-ground biomass. Shallow-rooting species might be more affected by drought than deep-rooting species for example.

Ecotype-Specific Reactions to Climate Manipulations

All species showed significant differences between ecotypes and at least one significant interaction with the drought or the warming manipulations per species. This implies that some ecotypes can cope significantly better with climate perturbations than others. Remarkably, our hypothesis that at least one southern ecotype would be better adapted to drought than the local ecotype could not be supported. The local ecotype did not fare significantly worse than the best-performing ecotype for any species or response parameter.

We found differences in the drought response of widespread species that can mainly be attributed to intraspecific variability. Climatic envelope models that are based on the whole range of species distribution may over-estimate the plasticity and adaptation capacity of plant species in regional species pools. However, general geographic trends or patterns cannot be traced on the basis of a small subset of ecotypes.

Additionally, our data suggest that in some species, ecological differences between ecotypes are more important than in others. This was mainly true for biomass and necrotic tissue for the first harvest after drought. At the second harvest, even more significant ecotype–drought interactions were found (Table 2). Here, some ecotypes showed the ability of compensatory growth after the drought while others did not (Figure 3b).

Implications for Adaptation Strategies

Climatic trends and especially extreme climatic events pose novel challenges to nature conservation (McClanahan *et al.* 2008; Felton *et al.* 2009). The conservation of managed grasslands in the context of climate change requires early and concerted action because of the longevity of key species, their clonal nature and the lack of direct control of species composition under traditional management. Populations that are composed of a wide variety of genotypes can be expected to also include plants that are adapted to locally novel climatic conditions (Bischoff *et al.* 2010). Translating the insurance hypothesis (Yachi & Loreau 1999) to the level of populations and phenotypic diversity, it can be expected that phenotypically mixed populations could better support the maintenance of ecosystem functions in the face of a higher probability of climatic extremes. Our results suggest that

assisted colonization of ecotypes (Kreyling *et al*. 2011) could increase the adaptive potential of populations. The positive and negative aspects of establishing populations with enhanced genetic diversity that are represented in the broader range of a species' distribution area than the one covered by local populations, however, must be discussed. One possibility is that local gene pools could be eliminated (McKay *et al.* 2005). Furthermore, the sensitivity of ecotypes to herbivores and other biotic interactions must also be investigated.

Nevertheless, for key species, which are responsible for maintaining ecosystem functioning, such as the ones considered in this study, a screening for better adapted ecotypes seems to be a promising tool to ensure the ecosystem performance and functionality. Climate change is a moving target (Jump & Penuelas 2005), and what has been considered to be natural and adapted until now, might not necessarily be so in the near future (Chiarucci *et al.* 2010).

In our study we focused on drought and warming as major stressors of plants. However, other kinds of extreme weather such as periods of heavy rain, hail storms, heat waves and late frost events are also expected to become more frequent and more intense. Therefore, combined effects between climatic trends and extremes and the contribution of within-species variability need to be further investigated.

Conclusions

It can be expected that the overall performance of ecosystems is largely dependent on the functioning of key species. This applies to the grass species of this study, which are able to contribute substantially to the productivity of European grassland communities.

We conclude that projections of ecological consequences of climatic extremes have to consider within-species variability besides biodiversity, community composition and species identity. If the environment is modified, as it is in the context of climate change, then either evolutionary processes of an uncertain duration will be stimulated or species will be replaced by better competitors. In the case of permanent grasslands, which are dominated by clonal species, inertia regarding to the responses within and between species can be expected.

The replacement of local phenotypes by apparently better adapted members of distant populations from the same species is unlikely to happen by natural means because the species' ecological niche is already occupied by a remnant population of the same species. Even if this remnant population was no longer vital (e.g. no longer reproducing) in perennial clonal species, the legacy of historic establishments would be sustained for a long period of

time. This could then delay the immigration and establishment of potentially better-adapted phenotypes of the same species. Our results on selected ecotypes of key grass species in managed European grasslands imply that the debate for and against ‘assisted migration’ has to be extended to the translocation of phenotypes. Obviously, we need more large-scale provenance and ecotype trials for the common species of permanent grasslands.

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References

Beier, C., Emmett, B., Gundersen, P., Tietema, A., Penuelas, J., Estiarte, M., Gordon, C., Gorissen, A., Llorens, L., Roda, F. & Williams, D. (2004) Novel approaches to study climate change effects on terrestrial ecosystems in the field: Drought and passive nighttime warming. *Ecosystems*, **7**, 583-597.

Beniston, M. & Stephenson, D.B. (2004) Extreme climatic events and their evolution under changing climatic conditions. *Global and Planetary Change*, **44**, 1-9.

BfG (2009) REMO-BFG version 5.8. Max Planck Institute for Meteorology on behalf of the Bundesanstalt für Gewässerkunde (BfG).

Bischoff, A., Steinger, T. & Mueller-Schaerer, H. (2010) The importance of plant provenance and genotypic diversity of seed material used for ecological restoration. *Restoration Ecology*, **18**, 338–348.

Breshears, D.D., Cobb, N.S., Rich, P.M., Price, K.P., Allen, C.D., Balice, R.G., Romme, W.H., Kastens, J.H., Floyd, M.L., Belnap, J., Anderson, J.J., Myers, O.B. & Meyer, C.W. (2005) Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences - USA*, **102**, 15144–15148.

Chiarucci, A., Araújo, M.B., Decocq, G., Beierkuhnlein, C. & Fernández-Palacios, J.M. (2010) The concept of Potential Natural Vegetation: an epitaph? *Journal of Vegetation Science*, **21**, 1172-1178.

Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogee, J., Allard, V., Aubinet, M., Buchmann, N., Bernhofer, C., Carrara, A., Chevallier, F., Noblet, N. de, Friend, A.D., Friedlingstein, P., Grunwald, T., Heinesch, B., Keronen, P., Knohl, A., Krinner, G., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourcival, J.M., Papale, D., Pilegaard, K., Rambal, S., Seufert, G., Soussana, J.F., Sanz, M.J., Schulze, E.D., Vesala, T. & Valentini, R. (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature*, **437**, 529–533.

Clark, R.T., Murphy, J.M. & Brown S.J. (2010) Do global warming targets limit heatwave risk? *Geophysical Research Letters*, **37**, L17703.

EUROSTAT (2010) http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/

Faraway, J.J. (2006) Extending the linear model with R-generalized linear, mixed effects and nonparametric regression models. Chapman & Hall/CRC, Boca Raton, 301 pp.

Fay, P.A., Kaufman, D.M., Nippert, J.B., Carlisle, J.D. & Harper, C.W. (2008) Changes in grassland ecosystem function due to extreme rainfall events: implications for responses to climate change. *Global Change Biology*, **14**, 1600-1608.

Felton, A., Fischer, J., Lindenmayer, D.B., Montague-Drake, R., Lowe, A.R., Saunders, D., Felton, A.M., Steffen, W., Munro, N.T., Youngentob, K., Gillen, J., Gibbons, P., Bruzgul, J.E., Fazey, I., Bond, S.J., Elliott, C.P., Macdonald, B.T., Porfirio, L.L. Westgate, M. & Worthy, M. (2009) Climate change, conservation and management: an assessment of the peer-reviewed scientific journal literature. *Biodiversity and Conservation*, **18**, 2243-2253.

Fetcher, N. & Shaver, G.R. (1990) Environmental sensitivity of ecotypes as a potential influence on primary productivity. *American Naturalist*, **136**, 126-131.

Gutschick, V.P. & BassiriRad, H. (2003) Extreme events as shaping physiology, ecology, and evolution of plants: toward a unified definition and evaluation of their consequences. *New Phytologist*, **160**, 21-42.

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G., Finn, J.A., Freitas, H., Giller, P.S., Good, J., Harris, R., Hogberg, P., Huss-Danell, K., Joshi, J., Jumpponen, A., Korner, C., Leadley, P.W., Loreau, M., Minns, A, Mulder, C.P.H., O'Donovan, G., Otway, S.J., Pereira, J.S., Prinz A., Read D.J., Scherer-Lorenzen, M., Schulze, E.D., Siamantziouras, A.S.D., Spehn, E.M., Terry, A.C., Troumbis, A.Y., Woodward, F.I., Yachi, S. & Lawton, J.H. (1999) Plant diversity and productivity experiments in European grasslands. *Science*, **286**, 1123-1127.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology.*, **25**, 1965-1978.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Hufford, K.M. & Mazer, S.J. (2003) Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology and Evolution*, 18, 147–155.

Humphreys, M.W., Yadav, R.S., Cairns, A.J., Turner, L.B., Humphreys, J. & Skot, L. (2006) A changing climate for grassland research. *New Phytologist*, **169**, 9-26.

IPCC (2007) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Jentsch, A. & Beierkuhnlein C. (2008) Research frontiers in climate change: Effects of extreme meteorological events on ecosystems. *Comptes Rendus Geoscience*, **340**, 621-628.

Jentsch, A. & Beierkuhnlein, C. (2010) Simulating the future responses of ecosystems, key species and European provenances to expected climatic trends and events. *Nova Acta Leopoldina NF*, **112** (384), 89-98.

Jentsch, A. (2006) Extreme climatic events in ecological research. *Frontiers in Ecology and the Environment*, **4**, 235-236.

Jentsch, A., Kreyling, J. & Beierkuhnlein, C. (2007) A new generation of climate change experiments: events, not trends. *Frontiers in Ecology and the Environment*, **5**, 365-374.

Jentsch, A., Kreyling, J., Boettcher-Treschkow, J. & Beierkuhnlein, C. (2009) Beyond gradual warming: extreme weather events alter flower phenology of European grassland and heath species. *Global Change Biology*, **15**, 837-849.

Jentsch, A., Kreyling, J., Elmer, M., Gellesch, E., Glaser, B., Grant, K., Hein, R., Lara, M., Mirzaei, H., Nadler, S.E., Nagy, L., Otieno, D., Pritsch, K., Rascher, U., Schadler, M., Schloter, M., Singh, G.K., Stadler, J., Walter, J., Wellstein, C., Woellecke, J. & Beierkuhnlein, C. (2011) Climate extremes initiate ecosystem regulating functions while maintaining productivity. *Journal of Ecology*, **Special Issue Extreme Events**.

Jump, A.S. & Penuelas, J. (2005) Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters*, **8**(9), 1010-1020.

Knapp, A.K., Beier, C., Briske, D.D., Classen, A.T., Luo, Y., Reichstein, M., Smith, M.D., Smith, S.D., Bell, J.E., Fay, P.A., Heisler, J.L., Leavitt, S.W., Sherry, R.A., Smith, B. & Weng, E. (2008) Consequences of more extreme precipitation regimes for terrestrial ecosystems. *Bioscience*, **58**, 811-821.

Kreyling, J., Bittner, T., Jaeschke, A., Jentsch, A,Steinbauer, M., Thiel, D., Beierkuhnlein, C. (2011). Assisted colonization. A question of focal units and recipient localities. Restoration Ecology 19: 433-440.

Kreyling, J., Wenigmann, M., Beierkuhnlein, C. & Jentsch, A. (2008) Effects of extreme weather events on plant productivity and tissue die-back are modified by community composition. *Ecosystems*, **11**, 752-763.

Macel, M., Lawson, C.S., Mortimer, S.R., Smilauerova, M., Bischoff, A., Cremieux, L., Dolezal, J., Edwards, A.R., Lanta, V., Bezemer, T.M., van der Putten, W.H., Igual, J.M., Rodriguez-Barrueco, C., Mueller-Schaerer, H. & Steinger, T. (2007) Climate vs. soil factors in local adaptation of two common plant species. *Ecology*, **88**, 424–433.

McClanahan, T.R., Cinner, J.E., Maina, J., Graham, N.A.J., Daw, T.M., Stead, S.M., Wamukota, A., Brown, K., Ateweberhan, M., Venus, V. & Polunin, N.V.C. (2008) Conservation action in a changing climate. *Conservation Letters*, **1**, 53-59.

McKay, J.K., Christian, C.E., Harrison, S. & Rice, K.J. (2005) “How local is local?" - A review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology*, **13**, 432–440.

Meehl, G.A., Karl, T., Easterling, D.R., Changnon, S., Pielke, R., Changnon, D., Evans, J., Groisman, P.Y., Knutson, T.R., Kunkel, K.E., Mearns, L.O., Parmesan, C., Pulwarty, R., Root, T., Sylves, R.T., Whetton, P. & Zwiers, F. (2000) An introduction to trends in extreme weather and climate events: Observations, socioeconomic impacts, terrestrial ecological impacts, and model projections. *Bulletin of the American Meteorological Society*, **81**, 413-416.

Michalski, S.G., Durka, W., Jentsch, A.,Kreyling, J., Pompe, S., Willner, E. & Beierkuhnlein, C. (2010) Evidence for genetic differentiation and divergent selection in an autotetraploid forage grass (*Arrhenatherum elatius*). *Theoretical and Applied Genetics*, **120**, 1151-1162.

Mirzaei, H., Kreyling, J., Hussain, M.Z., Li, Y., Tenhunen, J., Beierkuhnlein, C. & Jentsch, A. (2008) A single drought event of 100-year recurrence enhances subsequent carbon uptake and changes carbon allocation in experimental grassland communities. *Journal of Plant Nutrition and Soil Science*, **171**, 681-689.

Morecroft, M.D., Masters, G.J., Brown, V.K., Clarke, I. P., Taylor, M.E. & Whitehouse, A.T. (2004) Changing precipitation patterns alter plant community dynamics and succession in an ex-arable grassland. *Functional Ecology*, **18**, 648-655.

Oberdorfer, E. (2001) Pflanzensoziologische Exkursionsflora. Eugen Ulmer Verlag, Stuttgart.

Ofir, M. & Kigel, J. (2010) Ecotypic variation of summer dormancy relaxation associated with rainfall gradient in the geophytic grass Poa bulbosa. *Annals of Botany*, **105**, 617–625.

O’Gormann, P.A. & Schneider, T. (2009) The physical basis for increases in precipitation extremes in simulations of the 21st-century climate change. *Proceedings of the National Academy of Sciences - USA,* **106**, 14773-14777.

Pinheiro, J.C. & Bates, D.M. (2004) Mixed-effects models in S and S-PLUS. Springer, New York.

Ponsens, J., Hanson, J., Schellberg, J. & Moeseler, B.M. (2010) Characterization of phenotypic diversity, yield and response to drought stress in a collection of Rhodes grass (Chloris gayana Kunth) accessions. *Field Crops Research*, **118**, 57–72.

R Development Core Team. 2010. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. [http://www.R-project.org].

Reusch, T.B.H., Ehlers, A., Hammerli, A. & Worm, B. (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences - USA*, **102**, 2826-2831.

Rowell, D.P. (2008) Projected midlatitude continental summer drying: North America and Europe. *Journal of Climate*, **22**, 2813-2833.

Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J.H.C. & Gurevitch, J. (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, **126**, 543-562.

Ryser, P. & Aeschlimann, U. (1999) Proportional dry-mass content as an underlying trait for the variation in relative growth rate among 22 Eurasian populations of *Dactylis glomerata* s.l. *Functional Ecology*, **13**, 473-482.

Sillmann, J. & Roeckner, E. (2008): Indices for extreme events in projections of anthropogenic climate change. *Climatic Change*, **86**, 83-104.

Smith, J.B. Schneider, S.H., Oppenheimer, M., Yohe, G.W., Hare, W., Mastrandrea, M.D., Patwardhan, A., Burton, I., Corfee-Morlot J, Magadza, C.H.D., Fuessel, H.M., Pittock, A.B., Rahman, A., Suarez, A. & van Ypersele, J.P. (2009) Assessing dangerous climate change through an update of the Intergovernmental Panel on Climate Change (IPCC) ‘‘reasons for concern’’. *Proceedings of the National Academy of Sciences - USA*, **106**, 4133-4137.

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.-M., Hoegh-Guldberg, O. & Bairlein, F. (2002) Ecological responses to recent climate change. *Nature*, **416**, 389-395.

Yachi, S. & Loreau, M. (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proceedings of the National Academy of Sciences - USA*, **96**, 1463-1468.

6.5. Manuskript 5:

Cold hardiness of Pinus nigra Arnold as influenced by geographic origin, warming, and extreme summer drought

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frost hardiness, black pine, ecotype, cold tolerance, global warming, winter ecology

Abstract

Adaptation to the adverse effects of climate change is being investigated more and more through the introduction of species from warmer and drier climates, such as the (sub-) mediterranean *Pinus nigra* to dry sites in temperate Central Europe. Winter survival, however, may pose a serious threat to this strategy as cold extremes, which naturally determine the poleward range limits of forest trees, are not expected to follow the general warming trend in the near future.

Here, juveniles of *P. nigra* from eight provenances throughout Europe were exposed to different climate change scenarios (factorial combinations of 42 days of drought and warming by 1.6°C) in a common garden experiment in Bayreuth, Germany. Cold hardiness (LT50) was determined by the Relative Electrolyte Leakage method (REL) in two consecutive winters.

Cold hardiness of foliage differed by 10°C between the provenances studied and a local adaptation to minimum temperature was found. Cold hardiness was further affected by extreme summer drought, increasing cold hardiness by 3.9°C on average in the subsequent winter, and by summer warming, increasing cold hardiness by 3.4°C. Year-round warming had no significant effect on cold hardiness. Cold hardiness was related to the content of soluble carbohydrates and to the composition of fatty acids and alkanes in the needles.

Juveniles of *P. nigra* exhibited a comparable cold hardiness as juveniles of species native to Central Europe (*P. sylvestris, Picea abies, Fagus sylvatica* and *Quercus petraea*) under the same climatic conditions. Cold hardiness of the fine roots of *P. nigra* averaged -16.5°C compared to -23.8°C on average for needles.

Our results imply that the cold hardiness of the foliage is adaptive to both long-term growing conditions at the seed origin (genetic heritage) and short-term alterations of these conditions (individual plasticity), while first hints suggest that cold hardiness of the roots is high and might not be adaptive. For *P. nigra*, below- and above-ground cold hardiness of selected provenances in mid-winter appear suitable for cultivation in temperate regions.

Introduction

Species respond to climate change by poleward range shifts (Parmesan and Yohe, 2003). The speed of warming, however, is expected to exceed natural migration rates in many cases (Thomas et al., 2004). In forestry in particular, human-assisted range shifts are proposed to counter long generation cycles and modest dispersal abilities of forest trees (Schaberg et al., 2008b; McKenney et al., 2009). Yet, the importance of winter conditions is often overlooked, especially in the ecology of temperate regions (Kreyling, 2010). Absolute minimum temperatures have strong implications for species distributions by often determining their poleward range limits (Sakai and Weiser, 1973; Repo et al., 2008). A single cold extreme can offset any distributional adaptations to the general warming trend (Jalili et al., 2010) and in spite of the mean warming and their decreased frequency of occurrence, both the intensity and the duration of such cold extremes may even increase regionally within this century due to atmospheric circulation changes and internal atmospheric variability which counteract the warming trend from greenhouse forcing (Vavrus et al., 2006; Kodra et al., 2011).

Phenotypic plasticity and the adaptive potential of forest trees are determined by their high genetic diversity, allowing forest trees to develop local adaptations to environmental stressors (Hosius et al., 2006; Schaberg et al., 2008b). The cold hardiness of *Pinus devoniana*, for instance, increases with increasing frost risk along an altitudinal gradient (Saenz-Romero and Tapia-Olivares, 2008). Similarly, changes to the cold hardiness of *Fagus sylvatica* indicate local adaptation to the prevailing minimum winter temperatures (Visnjic and Dohrenbusch, 2004) and to late spring frost risk (Kreyling et al., 2011b) across Europe, and the frost tolerance of *Tsuga heterophylla* is adapted to frost risk along latitudinal and altitudinal gradients in North America (Kuser and Ching, 1980). Provenance trials demonstrate a differential performance between the provenances of different geographic origins of *Pinus*

nigra (Varelides et al., 2001), which is the target species of this study. *P. nigra* was selected because it is discussed in forestry as target species for translocations to Central Europe (Kölling, 2007; Huber 2011) and because of its high genetic diversity (Nkongolo et al., 2002; Jagielska et al., 2007). Based on its fragmented submediterranean range, one could assume that it lacks adaptation to winter frost, at least in some provenances. Provenance trials suggest that frost damage occurs around -20°C and in particular those provenances from Corsica do not survive -25°C (summarized in Huber, 2011).

The cold hardiness of evergreen tree species fluctuates over the course of the year. During acclimation in autumn, the plant organs become increasingly tolerant to the damaging effects of tissue freezing, particularly protecting cellular membranes which are a prime place of freezing injury (Bigras et al., 2001). Even though the genetic controls of the protective processes in conifers are complex and not yet sufficiently understood (Holliday et al., 2008), data has been summarized on the chemical components that are involved (Thomashow, 1999). During acclimation, lipid composition in the plasma membrane shifts towards more unsaturated lipids (Bakht et al., 2006) in addition to accumulation of soluble carbohydrates, hydrophilic polypeptides, antioxidants and chaperones in the membranes (Thomashow, 1999). Increased concentrations of all these chemical components serve the general purpose of preventing intra-cellular ice crystallization (Bigras et al., 2001).

Plants grown under generally warmer conditions, however, may lose their functional adaptations to frost (Eccel et al., 2009). Plants can further cope with different environmental stressors by similar responses at the cellular and molecular level when these stressors trigger similar signal chains. Drought and frost, for instance, lead to similar physiological responses in a coniferous forest tree - aiming to prevent cellular dehydration (Blodner et al., 2005). More frequent drought events may therefore make up for diminished acclimation due to warming.

The (sub-) mediterranean distribution of our target species *P. nigra* is reflected in high drought tolerance (Isajev et al., 2004) relative to temperate species such as *Pinus sylvestris* or *Fagus sylvatica*. Therefore, translocation of *P. nigra* is discussed as one adaptation strategy against the adverse effects of climate change at dry sites in Central Europe (Kölling, 2007). The minimum temperature in winter, however, is one of the most important factors setting the northern boundaries of the natural ranges of forest tree species (Sakai and Weiser, 1973; Koerner and Paulsen, 2004). The cold hardiness of one single provenance of *P. nigra* was lowest among eight *Pinus* species (with *P. nigra* showing the southernmost native range of the tested species) in a common garden experiment in Trondheim, Norway (Strimbeck et al.,

2007). As tree species are generally well adapted to the minimum temperatures of their environment (Sakai and Weiser, 1973), the range of frost tolerance of *P. nigra* across provenances needs to be examined in detail before translocations to other climates are undertaken. This holds particularly true because climate modelling implies that cold extremes will remain stable in their magnitude throughout this century in spite of climate warming (Vavrus et al., 2006; Kodra et al., 2011). With regard to the life span of trees, the expected decrease in frequency of cold spells (e.g. Vavrus et al., 2006; Kodra et al., 2011) is clearly less important than magnitude and duration of individual cold spells, as even with decreased frequency the likelihood of experiencing at least one cold spell is still close to 100%. Furthermore, forests grow slowly and management action aiming at stable and productive forests in future need to be started now. Target species for translocations need therefore not only be adapted to future conditions, but also survive current conditions with prevailing occurrences of cold extremes.

Plant organs differ in their cold hardiness. Generally, roots are the least frost tolerant (Mancuso, 2000; Bigras et al., 2001). The on-going decline of *Chamaecyparis nootkatensis* in the Pacific Northwest of North America, for instance, has been linked to root frost damage due to climate change-induced reductions in the insulating snow cover (Schaberg et al., 2008a). A similar reduction in snow cover is also projected for Central Europe (Kreyling and Henry, 2011). In addition to shoot cold hardiness, root freezing tolerance should therefore be investigated.

Here, eight provenances of *P. nigra* from autochthonous origins and from southern Germany were tested for their cold hardiness in a common garden experiment in southern Germany. We hypothesized that (1) cold hardiness differs between provenances, with provenances from colder origins displaying superior cold hardiness, and that (2) cold hardiness is affected by climatic experiences of the individuals with drought increasing cold hardiness and warming decreasing cold hardiness. We further expected that (3) differences in cold hardiness between provenances are physiologically-related to the content of soluble carbohydrates and lipid composition of the needles, and that (4) the (sub-) mediterranean species *P. nigra* is less frost-tolerant than tree species native to Central Europe, while (5) cold hardiness of the fine roots of *P. nigra* is high compared to cold hardiness of its foliage as it naturally occurs in regions without continuous snow cover.

Material and Methods

Juveniles of *P. nigra* from eight provenances throughout Europe were exposed to different climate change scenarios (warming and extreme drought) in a common garden experiment. Cold hardiness was determined by the Relative Electrolyte Leakage method (REL) in two consecutive winters. The experiment was established in Bayreuth, Germany (49°55'19" N, 11°34'55" E) in March 2009. The long-term mean annual temperature for the site is 8.2°C, whereas long-term mean annual precipitation is 724 mm.

Experimental Design

Eight provenances of *P. nigra* (Figure 1; Table 1) were obtained as seeds and cultivated at the *Bavarian Institute for Forest Seeding and Planting (ASP)* in Teisendorf, Germany from April 2008 to April 2009. These provenances are part of an international long-term provenance trial which started in 2009 (Huber, 2011). The provenances stem from autochthonous populations of *P. nigra* except for the provenance from Zellingen, Germany, which was introduced from Austrian sources in 1909. Subspecies identities of the provenances are assigned geographically and morphologically (Table 1), as genetic analyses are not yet available (Huber, 2011). The Croatian provenance stems from a location very close to one of the few autochthonous stands of *P. nigra* subspecies *dalmatica* and its assignment to the subspecies *nigra* is somewhat questionable. The seedlings were transported to Bayreuth and individually planted into 4-litre plastic pots filled with sandy silt (pH 7.3, total C 1.9%, total N 0.15%, plant available NO_3^--N 22.5 mg l^{-1}; plant available NH_4^+-N 1.8 mg l^{-1}). Selection of the plants occurred randomly for each provenance from all those plants alive at the planting date. The mean plant size at the start of the experiment was 12.2 cm ± 2.5 cm SD.

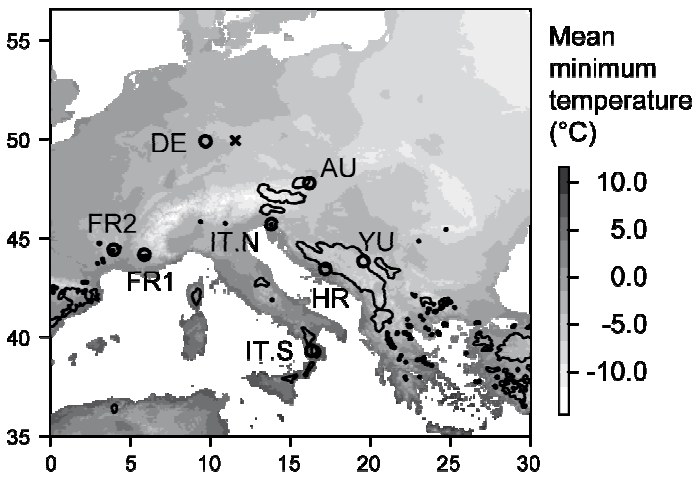

Figure 1: Origins of the target provenances (open circles) within the distribution of *P. nigra* (black lines and dots for fragmented populations Isajev et al., 2004). X indicates the experimental site. Abbreviations of provenances are specified in Table 1. Grey scales display the mean minimum temperature for the period 1950 to 2000 with a 5' spatial resolution (Hijmans et al., 2005).

Table 1: Origins of target provenances used in the experiment with corresponding climatic information. Skie: Identification number in an international provenance trial (Huber, 2011). MAT: Mean Annual Temperature; MinT: Mean Minimum Temperature; MAP: Mean Annual Precipitation; Precip. Seasonality: Coefficient of variation in mean monthly precipitation sum. All climate data for the period 1950 to 2000 from worldclim (Hijmans et al., 2005).

Provenance	Country	Subspecies	Skie	North	East	Altitude (m)	MAT (°C)	MinT (°C)	MAP (mm)	Precip. Seasonality
DE	Germany	*nigra*	01	49°53'17"	09°43'16"	290	9.2	-3.1	587	18
AU	Austria	*nigra*	07	47°46'00"	16°11'00"	369	8.4	-4.9	712	33
YU	Serbia	*nigra*	12	43°49'39"	19°35'22"	866	8.7	-5.6	964	17
HR	Croatia	*nigra/ dalmatica*	14	43°26'00"	17°13'00"	256	13.2	1.2	1108	33
IT.N	Italy	*nigra*	17	45°42'00"	13°49'00"	372	11.4	-1.2	1212	17
IT.S	Italy	*laricio*	19	39°18'08"	16°20'22"	1500	9.0	2.2	1300	48
FR1	France	*nigra*	23	44°09'10"	05°52'30"	549	10.7	-2.9	789	16
FR2	France	*laricio*	24	44°24'18"	03°58'39"	581	10.8	-0.9	745	19

The potted individuals were exposed to the fully crossed threefold factorial combination of (1) a drought manipulation (drought and control) and (2) a continuous warming manipulation (warming and reference) and (3) the provenance treatment (eight provenances). The two climate treatments were crossed resulting in four climate manipulations (control, drought, warming, warming & drought), that were replicated three times, resulting in 12 experimental units in total. The provenance treatment was nested within each experimental unit. Each provenance was further replicated with seven plants per experimental unit (nested replicates), resulting in 21 plants per factorial combination of the three-factorial design and 672 plants overall. Each experimental unit was covered by a single rain-out shelter (11 m by 7 m, 3.8 m high) constructed of a steel frame (GlasMetall Riemer GmbH) and covered with a transparent polyethylene sheet (0.2 mm, SPR5, Hermann Meyer GmbH) enabling an almost 90% penetration of photosynthetically-active radiation. The edge of the rain-out shelters was at a height of 80 cm.

The control irrigation regime simulated the local daily 30-year average precipitation. The application was carried out twice a week with collected rain water. The drought treatment consisted of 42 days without precipitation, which represents the local statistical 1000-year recurrence drought event. Drought duration was not *a priori* set before the manipulations. We monitored plant performance during the treatment and would have stopped the treatment

when either 66% of the plants showed water stress symptoms (discoloration of foliage) or when 33% of the plants exhibited lethal stress, or when the local 1000-year extreme would be reached. The latter condition was set because we assume that events with more than 1000 year recurrence time are not too realistic even when changing frequencies of extremes due to climate change are acknowledged (Schär et al., 2004). The same protocol was applied in a parallel experiment with four grass species (Beierkuhnlein et al., 2011), which all showed severe drought symptoms after about 20 days, emphasizing the high drought tolerance of *Pinus nigra*. The drought treatment started on May 27th 2009 and resulted in the soil moisture falling below the permanent wilting point (pF = 4.2) of the soil approximately three weeks after the start of the treatment (Figure 2). In the re-wetting phase each individual in the drought treatments received 240, 280 and 300 ml on three days within one week (in total 820 ml or 36 mm). Following that, the pots were irrigated according to the control precipitation treatment. Total amount of precipitation in the drought treatment was 13% lower than in the control over the year. The drought was simulated in the first year of the experiment only. Throughout the second year, all plants received control irrigation.

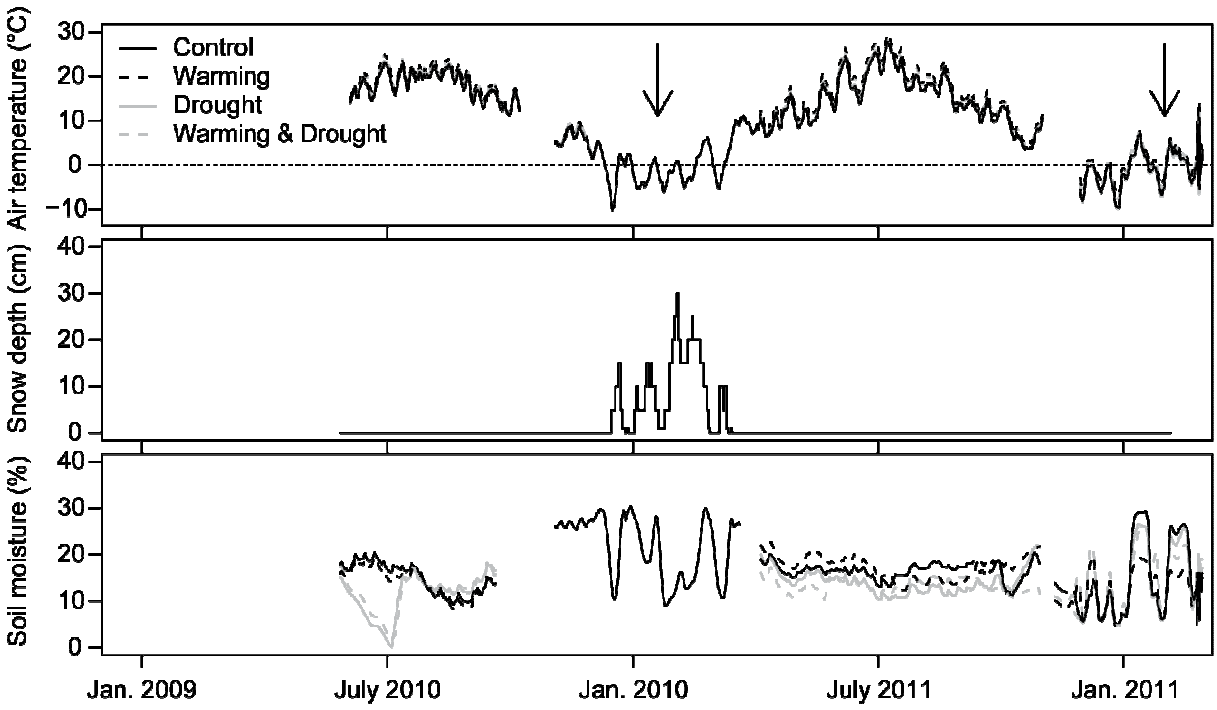

Figure 2: Temperature at mean plant height, snow cover and soil moisture (-2.5 to -7.5 cm) over the course of the experiment. Sampling dates are indicated by arrows.

The warming treatment was performed continuously until October in the first year of the experiment and from April to the end of the experiment in January of the second year. The warming manipulation took place both passively (wind-shelters which reduced the wind speed

by 70 % and black floor-covers versus white floor covers) and actively (IR-radiation with approximately 30 W per m²), which increased the air temperature at plant height by 1.6°C on average when the warming treatment was affected (Figure 2). Maximum differences were 5.2°C (single measurements) or 3.6°C for daily mean temperature. The fourth treatment was a combination of drought and warming. The warming increased the drought effect, reducing the soil moisture by another 1.5% on average (Figure 2).

During the first winter of the experiment, plants were kept outside the shelters in a sand bed from October to April. Figure 2 illustrates that the plants were covered by snow during the coldest parts of the first winter. For the second winter, plants were kept inside the shelters with the warming treatment ongoing.

Response Parameters

Cold hardiness was quantified by a slightly modified version of the relative electrolyte leakage method (REL) of ex-situ samples according to Strimbeck et al. (2007): Pre-tests revealed no differences in absolute values when the samples were frozen with or without 1ml solution containing an ice nucleator, presumably because the surface of the samples was wet and froze at around 0°C anyhow. Furthermore, higher freezing rates were applied. At a rate of 0.6°C/h (Strimbeck et al. 2007) it would have taken 3.5 days to reach our minimum temperature, whereas commonly the rate of 6°C/h is applied (e.g. Sutinen 1992, Schaberg 2008). Two needles from the current year were sampled per individual in mid-winter of both years (January 20th in 2010 and January 31st in 2011), rinsed with de-ionized water, and cut to 0.5 cm. Samples from the seven nested replicates per provenance and experimental unit were combined to form one mixed sample, homogenized and subsequently divided into seven subsamples subjected to different temperature levels for one hour (+4.5°C, -7.5°C, -14.5°C, -23°C, -33°C; -40°C, -196°C (liquid N)) using a controlled environment chamber (Licht-Thermostate Typ 1301,RUMED) and a manually controlled chain of freezers sequentially at the lowest temperatures. Initial electrolyte leakage was determined in 16 ml 0.1% v/v Triton X-100_Bidest after 24 h and the final electrolyte leakage was determined after autoclavation of the samples. Electrolyte leakage was quantified by the conductivity of the solution at 25°C measured with a WTW inolab pH/Cond 720. Cold hardiness is expressed as the LT50 for each mixed sample, estimated by non-linear regression of the REL versus the temperature levels using the formula by Anderson et al. (1988):

$$Y_T = Y_{min} + \frac{Y_{max} - Y_{min}}{1 + e^{k(T_m - T)}} \quad (1)$$

Y_T is the REL at temperature T, Y_{min} is the asymptotic value of the response variable in uninjured tissue, Y_{max} is the asymptotic value at maximum low-temperature stress, k represents the steepness of the response curve, and T_m is the midpoint of the symmetrical curve (an estimate of LT50). Curve fitting was carried out using a quantile regression and the function "nlrq()" from the software package "quantreg" (Koenker, 2006).

The multitude of different technical protocols for REL used in the literature (freezing with or without additional solution, various freezing rates and durations, etc.) limits the comparability between studies strongly. However, the relative differences within a protocol should be robust and more or less independent of e.g. freezing rates (Sutinen et al 1992). Therefore, we stick to the interpretation of relative differences within our study and minimize the discussion of absolute values.

Cold hardiness of needles from the current year or terminal buds was additionally determined for juvenile and adult individuals of the most important local tree species (*P. sylvestris, Picea abies, Fagus sylvatica* and *Quercus petraea*). Samples were taken on 26th of January in the first winter. Three mixed samples of seven individuals each were obtained from a nearby forest (lowland site: 350 m asl) and, for the juvenile stage of the two conifers, from a highland site (Waldstein, Fichtelgebirge, 760 m asl) about 50 km northwest of the experimental site.

In the second winter, carefully excavated fine roots of two provenances (FR1 and IT.S) exhibiting low and high cold hardiness in their foliage in the first year were analyzed for their cold hardiness by applying the same protocol as for the needles.

Mean annual minimum temperatures for the period 1950 to 2000 (mean temperature of the coldest day for the years 1950-2000) for each geographic origin of the provenances were retrieved from worldclim (Hijmans et al., 2005) and used as indicators for minimum temperatures (Table 1). We assume that the relative differences between geographic origins have been suitably reflected, although these values exceed the absolute minimum temperatures due to daily averaging (for our experimental site the minimum temperature based on worldclim is -3.5°C while the absolute annual minimum temperatures between 1998 and 2011 at an hourly resolution ranged between -10.8 and -25.5°C). An ordinary least squares regression between this indicator and cold hardiness was applied for the control treatment in order to detect local adaptation to late frost events.

Soluble carbohydrates were quantified in the first winter for two provenances exhibiting low and high cold hardiness, respectively (FR1 and IT.S). Mixed samples of one needle from the seven plants per experimental unit were taken, immediately frozen in liquid nitrogen and stored at -30°C. Frozen material was ground in a ball mill; soluble carbohydrates of 20 mg of plant material were extracted in 50 % methanol and analyzed using the anthrone method (Kleber et al., 1997). Extinction was measured at 620 nm. We used known concentrations of Glucose as a standard.

Lipid composition was obtained for the same two provenances (FR1 and IT.S). Epicuticular wax lipids including alkanes as the most abundant wax component were recovered by rinsing needles for 60 seconds in dichloromethane (DCM), which resembles standard techniques using chloroform (Radler and Horn, 1965). After removal of the epicuticular wax lipids, needles were ground to a fine powder using a ball mill (Retsch M200). Internal waxes were recovered by standard Soxhlet extraction using a mixture of DCM/Methanol (93:7) (Wiesenberg et al., 2010). Extracts of internal and epicuticular waxes were dried and sequentially separated using solid-phase extraction into lipid fractions including fatty acid and alkane fractions (Wiesenberg et al., 2010). The following section only discusses the results of the alkane fraction as a representative component of epicuticular wax lipids and the fatty acids of internal lipids as the dominant compound class of cell membranes. Other fractions were also analyzed, but no significant differences in their distribution patterns were obtained. Aliquots of deuteriated standards ($D_{39}C_{20}$ acids and $D_{50}C_{24}$ alkane, respectively) were added to the lipid fractions for compound identification and quantification. Fatty acid fractions were derivatized using BSTFA (N,O-Bis(trimethylsilyl)trifluoroacetamide) for 1h at 80°C, whereas alkanes did not require any further preparation. All fractions were measured using gas chromatography coupled with flame ionization detection (Agilent 7890).

In addition to lipid distribution patterns, molecular proxies were also determined to evaluate the differences between provenances and climate manipulations. The average chain length (ACL) of lipids is influenced by lipid biosynthesis and regulates the water repellency of hydrophobic hydrocarbon chains of fatty acids in cell membranes as well as fatty acids and alkanes in epicuticular waxes (Kolattukudy et al., 1976). Initially, the ACL was used to differentiate plant and microbial sources of organic matter in terrestrial sediments (Bray and Evans, 1961):

$$ACL = \Sigma (z_n * n) / \Sigma (z_n) \quad (2)$$

where n is the number of carbons and z_n the amount of fatty acids or alkanes with n carbons. Another parameter to obtain the differences in the lipid biosynthesis as affected by environmental stress is the predominance of odd versus even alkanes, the so-called carbon preference index (CPI: Kolattukudy et al., 1976):

$$CPI = [(\Sigma\ n\text{-}C_{25\text{-}33\ odd} / \Sigma\ n\text{-}C_{24\text{-}32\ even}) + (\Sigma\ n\text{-}C_{25\text{-}33\ odd} / \Sigma\ n\text{-}C_{26\text{-}34\ even})]/2 \quad (3)$$

The degradation of alkanes and a less effective synthesis of the predominant odd alkanes in waxes lead to a reduction of the CPI under environmental stress (Wiesenberg et al., 2008).

An analysis of variance (ANOVA) combined with linear mixed effect models were applied to test for the main and interactive effects of the three factors: provenance, drought, and warming on cold hardiness (LT50), soluble carbohydrate content and lipid composition. Including the experimental unit as a random factor accounted for the split-plot design (Pinheiro and Bates, 2004). Data were log transformed to improve the homogeneity of variances and the normality of residuals if necessary. All statistical analyses were conducted with the software R 2.11.1 (R Development Core Team, 2010) and the additional packages “nlme” and “quantreg”.

Results

Local Adaptation in Cold Hardiness

Mean cold hardiness differed between the provenances by about 10°C in both winters (Figure 3). The LT50 values ranged between a minimum of -21.2°C for provenance FR2 and a maximum of -32.1°C for provenance FR1 in the first winter; and between a minimum of -23.2°C for provenance FR2 and a maximum of -33.1°C for the provenance from Serbia (YU) in the second winter. Provenances from colder origins generally displayed superior cold hardiness (Figure 4). Significant correlations ($r^2 = 0.77$ in the first winter and $r^2 = 0.80$ in the second winter) between cold hardiness and mean minimum winter temperature at the origins were found for the autochthonous provenances in both years if the provenance from Croatia was excluded from the analyses. When included, no significant correlation was found in the first winter, while the correlation was weaker ($r^2 = 0.59$) while still remaining significant in the second winter.

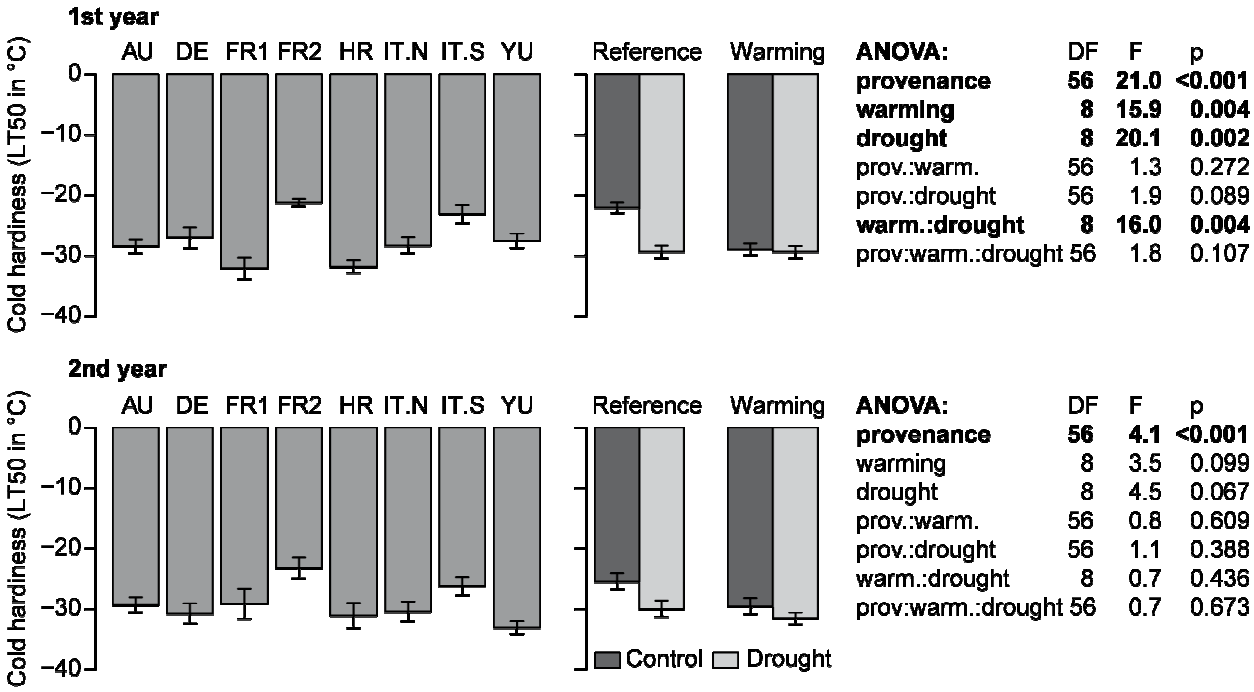

Figure 3: Cold hardiness (LT50) as affected by geographic origin (provenances; left) and preceding climatic conditions (drought and warming in interaction; right) in the first (upper panel) and second (lower panel) year of the experiment. ANOVA-results are provided per year with significant effects in bold. Mean values and standard errors are shown for 84 individuals per bar for the provenances and 168 individuals per bar for the climate treatments. Note that the drought manipulation only took place in the first summer of the experiment. The warming treatment stopped three months before sampling in the first year while running throughout sampling in the second year.

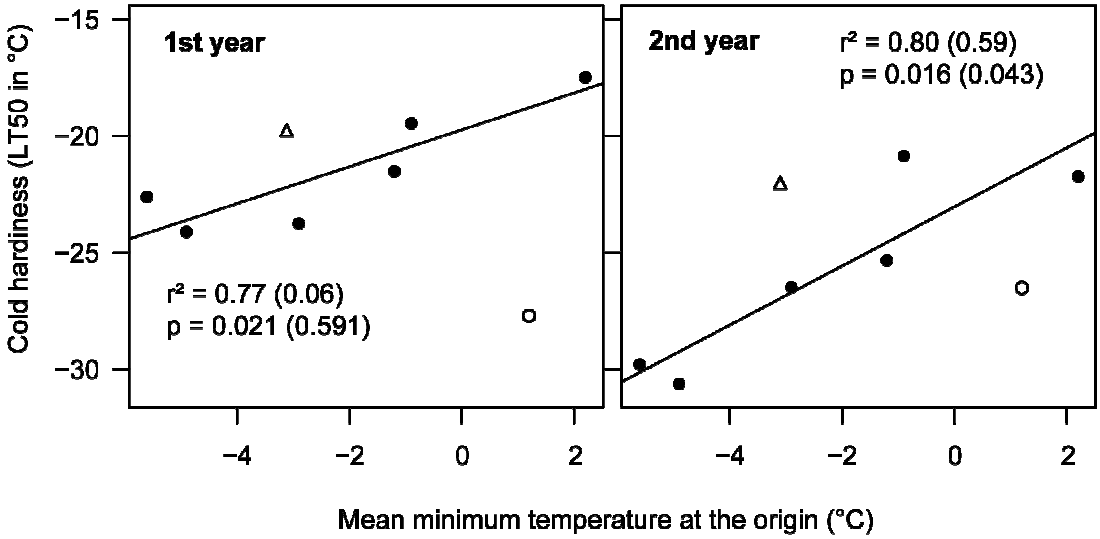

Figure 4: Local adaptation in cold hardiness depending on the mean minimum temperature at the origin. Linear regressions are shown for all autochthonous provenances (DE, open triangle, not included) excluding the provenance from Croatia (HR, open circle), as its autochthonous status is questionable. Results of the regression including the provenance from Croatia are given in parentheses. Cold hardiness (LT50) displays the mean of the control treatment per provenance (n = 21).

Climatic Experiences Alter Cold Hardiness

Cold hardiness was affected by the climatic experiences of the individuals. The extreme summer drought increased cold hardiness by 3.9°C on average in the first winter and there was a non-significant trend in the same direction in the second winter after the drought manipulation (Figure 3). Unexpectedly, the summer warming from the first year resulted in increased cold hardiness of 3.4°C on average while the year-round warming of the second year resulted in no significant effect, although the trend followed the same direction as in the first year. Interestingly, the drought and the warming effect in the first year were not additive (ANOVA, interaction between drought and warming: $F = 16.0$; $p = 0.004$), resulting in lower cold hardiness in the untreated variant and comparable, high cold hardiness in the other three climate manipulations (Figure 3).

Physiological Reasons for Varying Cold Hardiness

The amount of soluble carbohydrates in the needles increased by 25.9% in a provenance exhibiting high cold hardiness compared to a provenance showing low cold hardiness (Table 2; ANOVA: $F = 15.3$; $p = 0.004$). The drought manipulation had no significant effect on the carbohydrate concentration ($F = 0.0$; $p = 0.889$).

The composition of internal fatty acids (ACL) as main components of cell membranes did not differ significantly between a provenance exhibiting high cold hardiness and a provenance showing low cold hardiness ($F = 3.7$; $p = 0.092$). Likewise, no effect of the drought manipulation was found ($F = 1.7$; $p = 0.222$). For the epicuticular wax lipids, the provenance with the high cold hardiness was characterized by a slightly higher ACL (3.9 %; $F = 5.2$; $p = 0.051$) than that with the low cold hardiness. The drought treatment led to a general decrease in ACL ($F = 22.0$; $p = 0.002$), which was stronger for the plants with a high (5.5 %) rather than a low cold hardiness (1.5 %; interaction between provenance and drought manipulation: $F = 7.7$; $p = 0.024$).

The greatest differences among lipids were observed for the CPI values of the epicuticular wax alkanes between the two provenances. CPI values were 22.7 % lower for the provenance with high cold hardiness compared to that of the provenance with low cold hardiness ($F = 45.8$; $p < 0.001$). Drought led to an increase of the CPI value by 11.5 % in the provenance with high cold hardiness, while no effect of the drought manipulation was observed in the provenance with low cold hardiness, resulting in no significant effects of the drought manipulation ($F = 2.3$; $p = 0.166$) and the interaction between provenance and drought ($F =$

3.4; p = 0.104). Hence, the difference between both provenances decreased after drought, but CPI values were still 15.1 % higher in plants with low cold hardiness.

Table 2: Comparison of carbohydrate content and average chain length (ACL) of fatty acids of current year needles between two provenances exhibiting low (IT.S) and high (FR1) cold hardiness. Samples taken in the first winter of the experiment, mean ± standard deviation provided, n = 3.

	Cold hardiness of source	Control	Drought
Cold hardiness (LT50 in °C)	high	-25.8 ± 2.3	-36.7 ± 1.0
	low	-17.5 ± 1.6	-26.8 ± 4.5
Soluble carbohydrates (‰TM)	high	118.4 ± 9.4	111.9 ± 9.5
	low	89.0 ± 15.9	93.7 ± 3.0
ACL of epicuticular wax fatty acids	high	18.8 ± 0.2	17.7 ± 0.2
	low	18.1 ± 0.0	17.8 ± 0.1
ACL of internal fatty acids	high	17.8 ± 0.5	17.6 ± 0.1
	low	17.5 ± 0.1	17.1 ± 0.3
ACL of epicuticular wax alkanes	high	27.5 ± 0.1	27.6 ± 0.0
	low	27.5 ± 0.1	27.5 ± 0.1
CPI of epicuticular wax alkanes	high	9.3 ± 0.2	10.4 ± 0.2
	low	12.0 ± 0.6	11.9 ± 0.4

Cold Hardiness among Species

Cold hardiness of the (sub-) mediterranean *P. nigra* reached similar levels to the cold hardiness of tree species native to Central Europe in the vicinity of the experimental site (Figure 5, ANOVA for all juvenile lowland samples: F = 2.0; p = 0.163). Needles of adult conifers, however, showed superior cold hardiness compared to juvenile trees (F = 2.6; p = 0.046; *Picea abies* and *Pinus sylvestris*) and juveniles from highland sites exhibited higher cold hardiness compared to lowland sites (F = 7.4; p = 0.026; *Picea abies* and *Pinus sylvestris*).

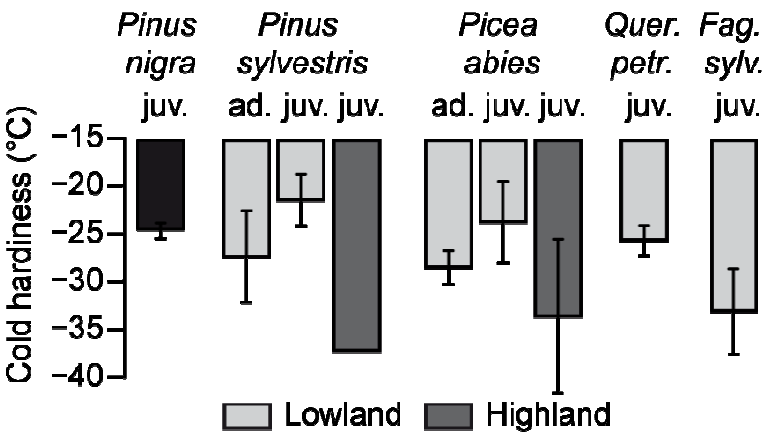

Figure 5: Comparison of the cold hardiness (LT50) of *P. nigra* with common forest tree species in the vicinity of the experimental site (lowland, 350 m asl) and, for the juvenile stage of the other two conifers, from a highland site (760 m asl). juv.: juveniles (2-4 years old); ad.: adults (>30 years old). *Quer. petr.: Quercus petraea; Fag. sylv.: Fagus sylvatica.* n = 3 mixed samples of 7 individuals each per bar (mean and SE). For *P.nigra*: provenance DE (Zellingen, Germany) in the control treatment.

Cold Hardiness of Roots

Cold hardiness of fine roots of *P. nigra* averaged -16.5°C. The two tested provenances (IT.S and FR1) did not differ significantly ($F = 1.6$; $p = 0.239$) in the cold hardiness of their roots in the second winter of the experiment (-15.4 ± 1.9°C and -17.5 ± 1.1°C respectively (±1SE), n = 12). The drought ($F = 0.1$; $p = 0.805$) and warming ($F = 0.1$; $p = 0.754$) treatments also resulted in no significant effect on LT50 of the fine roots.

Discussion

Local Adaptation in Cold Hardiness

Cold hardiness differed by about 10°C between the studied provenances of *P. nigra*. Local adaptation to minimum temperature regimes was indicated as provenances from colder origins reached superior cold hardiness. These results correspond well with findings from other forest trees such as *P. devoniana* (Saenz-Romero and Tapia-Olivares, 2008), *Fagus sylvatica* (Visnjic and Dohrenbusch, 2004), *Tsuga heterophylla* (Kuser and Ching, 1980), *Fagus crenata* and *Betula ermanii* (Gansert et al., 1999), all showing local adaptation to winter cold extremes. Our data indicates further that minimum temperature does not only determine the northern range limits of species (Sakai and Weiser, 1973), but that within species variability in cold hardiness also needs to be taken into account. The provenance from Croatia (HR), however, did not fit well into the overall pattern. We assume that this provenance is either not autochthonous, i.e. originating from a warmer winter climate, or

belongs to the subspecies *dalmatica*, which is described for very restricted areas along the Croatian coast. Genetic characterization of the species and subspecies will shed light on this question.

P. nigra is known for its high genetic diversity (Jagielska et al., 2007) which surpasses that of other pines (Nkongolo et al., 2002). Although no consensus on its taxonomy has been reached (Huber, 2011), six main subspecies are recognized with *P. nigra* ssp *nigra* being the most abundant in Europe (Isajev et al., 2004). Provenances furthermore differ in growth and ecological performance, expressed in local adaptations to soil and mean annual temperature and precipitation in provenance trials (Varelides et al., 2001). The strongly contrasting cold hardiness in our experiment suggests that minimum temperature is another genetically selective parameter, not only for frost sensitive subspecies such as *P. nigra* ssp *laricio* (Varelides et al., 2001), but also for *P. nigra* ssp *nigra*, which is usually considered to be the most frost tolerant among the subspecies (Isajev et al., 2004, Huber, 2011).

Climatic Experiences Alter Cold Hardiness

Cold hardiness was affected by climatic experiences of the individuals with drought increasing cold hardiness by 3.9°C on average in the subsequent winter and no significant carry-over effect to the second winter. This finding can be explained by drought and frost triggering similar responses at the cellular and molecular level to prevent cellular dehydration (Blodner et al., 2005). Without experiencing drought themselves, the newly formed needles in the second year of the experiment lacked significant additional cold hardiness in the drought manipulation. More frequent drought events accompanying climate change may therefore increase cold hardiness in single (dry) years, but not generally.

It has been suggested that trees grown under generally warmer conditions may lose their functional adaptations to frost (Eccel et al., 2009). Surprisingly, our results contradict this expectation with increased cold hardiness by 3.4°C on average in the warming treatment after the first season. The warming, however, was stopped in October and acclimation of the formerly warmed individuals evidently surpassed the control plants when subjected to the same temperature from October on. Responsiveness to current year climates are also reported for deciduous forest trees (Repo et al., 2008). Yet, the year-round warming in the second year of the experiment resulted in no significant difference between the treatments. Clearly, further experiments on interacting climatic drivers are urgently needed, as the response to such interactions might differ considerably from single factor experiments (Shaw et al., 2002; Kreyling et al., 2011b).

Here, we focused on the realized maximum frost hardiness, e.g. the hardiness directly after the coldest days of winter. Much bigger differences than observed between the provenances (10°C) or between the climate manipulations (up to 3.9°C) occur within each needle over the course of the year (more than 60°C in a single provenance of *P. nigra*; Sutinen et al., 1992). Cues which drive this strong seasonality involve photoperiod and minimum temperature experience. Their relative importance, however, is still unresolved, differs between species (Kozlowski and Pallardy, 2002; Holliday et al., 2008) and may even differ between provenances in the same species. Our results add to this discussion by showing that both genetic heritage (differences between the provenances) and preceding climatic experience (here mainly summer drought) can affect the absolute frost hardiness. Potential differences in the temporal pattern of frost hardiness between provenances are of high ecological relevance, especially with regard to early or late frost events and phenological differences within species (Visnjic and Dohrenbusch, 2004; Kreyling et al. 2011b). These points call for more detailed investigations on intra-specific differences in seasonality of frost hardiness.

Physiological Reasons for Different Cold Hardiness

Differential cold hardiness between provenances was related to contents of soluble carbohydrates and fatty acids in the needles. Content of soluble carbohydrates is also reported to be closely related to local adaptations in cold hardiness of different *Quercus* species (Morin et al., 2007). The lipid contents of *P. nigra* have been reported previously for mature trees and needles collected during late summer (Maffei et al., 2004). In contrast to these mature trees, where *n*-C_{29} and *n*-C_{31} alkanes contribute 2.2 % and 37.2 %, respectively, to total alkanes, the juveniles of different provenances in our study were all dominated by *n*-C_{29} alkane (36.3 ± 2.8 %) and lower contents of *n*-C_{31} alkane (9.6 ± 1.0 %). This difference is probably due to different needle and plant age when compared to the literature results, whereas differences between provenances are not likely as they did not differ in their relative contribution of *n*-C_{29} and *n*-C_{31} alkanes in our study. In general, the hydrophobicity of the waxes is improved under water and cold stress to protect plants against water loss by an increased turnover of wax components towards hydrophobic aliphatic compounds which is not necessarily related to shifts in the total amount of waxes (Shepherd and Griffiths, 2006). For trees, such investigations are still scarce and limited to selected tree species (e.g. for different *Picea* species: Cape and Percy, 1993; or *Pinus palustris*: Prior et al., 1997). Our observations of small changes in the lipid composition (ACL values) confirm minor influences of cold and

water stress on lipid biosynthesis, as described elsewhere (Cape and Percy, 1993; Shepherd and Griffiths, 2006). The low CPI values of epicuticular wax alkanes of the plants with high cold hardiness, however, indicate a strong biosynthesis rate associated by a production of byproducts and degradation products such as even alkanes. This increased production of wax components indicates the role of alkanes to improve the cold hardiness (Prior et al., 1997). The drought manipulation led to a reduction in the formation rates of alkanes (higher CPI) for the provenance with higher cold hardiness similar as observed for sesame plants (Kim et al., 2007). Hence, biosynthesis of epicuticular wax alkanes is influenced by water stress and appears to be related to cold hardiness in *P. nigra.* Freezing tolerance in plants is accompanied by lipid remodeling at the outer membrane (Moellering et al. 2010), another aspect fitting well to our data and indicating that the effect of changes in the lipid composition might be more important for cold hardening than previously assumed. It should be noted, though, that both the observed differences in cold hardiness and the differences in composition and concentrations of cell membrane compounds could be driven by other factors such as water stress over summer in the provenance with superior frost hardiness. Seasonality of precipitation and mean annual precipitation were three times lower at the origin of this provenance (Table 1). Further causal and functional analyses of frost hardiness and hardening are clearly required (Holliday et al., 2008).

Cold Hardiness among Species

Juveniles of the (sub-) mediterranean species *P. nigra* exhibited comparable cold hardiness as juveniles of species native to Central Europe in the vicinity of the experiment, i.e. under the same climatic conditions. Under colder conditions in Norway it has been shown that *P. nigra* is more sensitive to freezing injury than boreal conifers (Strimbeck et al., 2007). Its ability to adjust to prevailing climatic conditions therefore appears limited in comparison to boreal species such as *P. sylvestris* or *Picea abies*. Yet, under the same climatic conditions, these species did not differ from *P. nigra* in our study, implying that realized frost hardiness and potential frost hardiness need to be discussed separately. It should be emphasized here that within-species variation in cold hardiness, i.e. differences among provenances of *P. nigra* and differences between lowland and highland sites or juvenile and adult individuals for the other species clearly exceeded among-species variation at the juvenile stage. Generally, variation among species at the same site and under the same climatic conditions appears less important

than commonly assumed. Within-species variation and individual performance might be more relevant for forest ecology (Clark, 2010).

We used juvenile trees in their second to third year in this experiment. Our results concerning *P. sylvestris* and *Picea abies* confirm previous findings that seedlings are more sensitive against frost events than older trees (Bolte et al., 2007). However, the juvenile stage is of high importance for the natural regeneration of forest stands. Moreover, the high selective pressure of single extreme events such as frost or drought can reduce the genetic diversity of future stands (Hosius et al., 2006).

Cold Hardiness of Roots

Cold hardiness of fine roots of *P. nigra* averaged -16.5°C over two provenances, which is a high value compared to the cold hardiness of its foliage (-23.8°C on average for the control treatment). This might be an adaptation to the species' natural habitat where soil frost events occur more or less regularly as no snow cover insulates the soil against air temperature fluctuations (Kreyling, 2010). *Chamaecyparis nootkatensis* serves as an example of a forest tree from temperate rain-forests with low root cold hardiness (roots do not survive temperatures below -5°C) in response to deep snow cover in its natural habitat (Schaberg et al., 2008a). Winter climate change, however, is expected to lead to reduced snow cover and, in consequence of the reduced insulation, to colder soils despite the general air warming trend (Groffman et al., 2001). For Central Europe, a reduction in snow cover is already taking place, while minimum temperature of the soil may not decrease (Kreyling and Henry, 2011). Interestingly, no response in cold hardiness of fine roots occurred for the different climate treatments in our experiment. In addition, we investigated root cold hardiness for two provenances with strongly contrasting shoot cold hardiness and did not find significant differences in the roots. This supports Schaberg et al. (2008a) who conclude that no acclimation occurs in cold hardiness of fine roots. More detailed investigations concerning this aspect are clearly needed, especially with respect to the question if cold hardiness of roots lacks adaptive potential to changing climate conditions. For *P. nigra* our results imply that no selective pressure is expected as root cold tolerance is generally high.

Assisted Colonization

P. nigra, based on its ecology and natural distribution (Isajev et al., 2004), is well adapted to warmer and drier conditions expected for parts of Central Europe under climate change (Kölling, 2007, Huber, 2011). Here, we show that cold hardiness, at least of some provenances, is also no limitation for the use of this species in Central Europe even if cold extremes remain constant throughout this century (Vavrus et al., 2006; Kodra et al., 2011). Assisted colonization or transplantations are widely applied in forestry and may serve as adaptation strategy against adverse effects of climate change on ecosystem functioning (McKenney et al., 2009, Schaberg et al., 2008b). Numerous examples of failed transplantations (Zobel et al., 1987), however, warn against rushed action. The assisted colonization of pre-adapted ecotypes of key species within their current range is suggested to contribute to functional integrity of forest stands without the need to introduce exotic species with unknown risks (Kreyling et al., 2011a). Yet, naturally dominating tree species may lack pre-adapted ecotypes at their warm and dry range limits. Here, congeneric species from adjacent climates are preferable over other species. *P. sylvestris* and *P. nigra* may serve as an example, with the latter potentially replacing the former at warmest and driest sites of its range while maintaining ecosystem functioning. Our results suggest that cold hardiness is significantly related to climatic conditions at the origin of the provenances, implying that the selection of frost-tolerant provenances could be based on the current climatic conditions within the species ranges. However, our finding that climatic experiences within the life of single plants alter cold hardiness indicates that provenance trials under control conditions may be misleading under changing climatic mean and extreme conditions. The multitude of possible climatic variables to be selected for and uncertainties concerning future climates imply that the search for best-adapted provenances should not be the only strategy. In addition, management actions which promote genetic diversity (e.g. supporting natural regeneration and addition of genetically diverse material) are crucial as genetic diversity enables organisms to continue adapting and evolving to new conditions within one or several generation cycles (Hosius et al., 2006; Schaberg et al., 2008b). Furthermore, the role of herbivores and diseases under changing climate requires detailed investigations. For instance, a needle blight known as the “red band disease” (*Dothistroma septospora*) is reported to increase in importance over recent years in *P. nigra* (Isajev et al., 2004), a development that may be related to climate change (Watt et al., 2011).

Ultimately, tree species responses should be regarded in the context of populations under competitive pressure. The advantage of common garden experiments is that they can detect the spectrum of possible species-specific responses. Nevertheless, there is a need to test the

obtained results in communities where the competitive balance might amplify or buffer responses.

Conclusions

Cold hardiness of *Pinus nigra* foliage is highly variable between provenances and shows signs of local adaptation to prevailing minimum temperatures at the origin. Both severe drought events and summer warming can increase cold hardiness, indicating that the interaction of different climate parameters leads to unexpected results and that winter survival can be altered by climatic events during the growing season. Physiologically, cold hardiness is related to soluble carbohydrate content and lipid composition. Interestingly, variation of cold hardiness of the needles within the (sub-) mediterranean species *P. nigra* was higher than between this species and other species common to the temperate zone of Central Europe. Taken together, our results imply that the cold hardiness of the foliage of *P. nigra* is adaptive to long-term growing conditions at the origin (genetic heritage) and to short-term alterations of these conditions (individual plasticity), while first hints suggest that cold hardiness of the roots is high and probably not under selective pressure currently. Our data from mid-winter suggests that below- and above-ground cold hardiness of selected provenances appear to be well adapted to cultivation in temperate regions as an adaptation strategy against the adverse effects of climate change in dry habitats. However, with respect to late spring and early autumn frost events, the temporal pattern of frost hardiness with potential intra-specific differences should be investigated in more detail. Before translocations are recommended, further investigations are required, e.g. exploring the role of biotic interactions under changing climatic conditions. Generally, within-species diversity should be conserved at the species level and improved in anthropogenically founded stands in order to allow for adaption to climate change.

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References

Anderson, J.A., Kenna, M.P., Taliaferro, C.M., 1988. Cold hardiness of Midiron and Tifgreen bermudagrass. Hortscience 23, 748–750.

Bakht, J., Bano, A., Dominy, P., 2006. The role of abscisic acid and low temperature in chickpea (*Cicer arietinum*) cold tolerance. II. Effects on plasma membrane structure and function. J Exp Bot 57, 3707–3715.

Bigras, F.J., Ryyppo, A., Lindstrom, A., Sattin, E., 2001. Cold acclimation and deacclimation of shoots and roots of conifer seedlings. In: Bigras, F.J., Colombo, S.J. (Eds.), Conifer cold hardiness. Kluwer, Dordrecht, pp. 57–88.

Blodner, C., Skroppa, T., Johnsen, O., Polle, A., 2005. Freezing tolerance in two Norway spruce (*Picea abies* [L.] Karst.) progenies is physiologically correlated with drought tolerance. J Plant Physiol 162, 549–558.

Bolte, A., Czajkowski, T., Kompa, T., 2007. The north-eastern distribution range of European beech - a review. Forestry 80, 413–429.

Bray, E., Evans, E., 1961. Distribution of n-paraffins as a clue to the recognition of source beds. Geochim Cosmochim Acta 22, 2–15.

Cape, J.N., Percy, K.E., 1993. Environmental-influences on the development of spruce needle cuticles. New Phytol 125, 787–799.

Clark, J.S., 2010. Individuals and the variation needed for high species diversity in forest trees. Science 327, 1129–1132.

Eccel, E., Rea, R., Caffarra, A., Crisci, A., 2009. Risk of spring frost to apple production under future climate scenarios: the role of phenological acclimation. Int J Biometeorol 53, 273–286.

Gansert, D., Backes, K., Kakubari, Y., 1999. Altitudinal and seasonal variation of frost resistance of *Fagus crenata* and *Betula ermanii* along the Pacific slope of Mt. Fuji, Japan. J Ecol 87, 382–390.

Groffman, P.M., Driscoll, C.T., Fahey, T.J., Hardy, J.P., Fitzhugh, R.D., Tierney, G.L., 2001. Colder soils in a warmer world: A snow manipulation study in a northern hardwood forest ecosystem. Biogeochemistry 56, 135–150.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25, 1965–1978.

Holliday, J.A., Ralph, S.G., White, R., Bohlmann, J., Aitken, S.N., 2008. Global monitoring of autumn gene expression within and among phenotypically divergent populations of Sitka spruce (*Picea sitchensis*). New Phytol 178, 103–122.

Hosius, B., Leinemann, L., Konnert, M., Bergmann, F., 2006. Genetic aspects of forestry in the central Europe. Europ J Forest Res 125, 407–417.

Huber, G., 2011. Internationaler Bayerischer Schwarzkiefern-Herkunftsversuch 2009/2010 - Neue Tests für Schwarzkiefern-Herkünfte in Bayern im Hinblick auf den Klimawandel. Forstarchiv, in press.

Isajev, V., Fady, B., Semerci, H., Andonovski, V., 2004. EUFORGEN Technical Guidelines for genetic conservation and use for European black pine (*Pinus nigra*). International Plant Genetic Resources Institute, Rome, Italy.

Jagielska, A., Cwalina, M., Prus-Glowacki, W., 2007. Genetic diversity of the black pine‘s stands (*Pinus nigra*) Arn. Sylwan 151, 23–31.

Jalili, A., Jamzad, Z., Thompson, K., Araghi, M.K., Ashrafi, S., Hasaninejad, M., Panahi, P., Hooshang, N., Azadi, R., Tavakol, M.S., Palizdar, M., Rahmanpour, A., Farghadan, F., Mirhossaini, S.G., Parvaneh, K., 2010. Climate change, unpredictable cold waves and possible brakes on plant migration. Global Ecol Biogeogr 19, 642–648.

Kim, K.S., Park, S.H., Jenks, M.A., 2007. Changes in leaf cuticular waxes of sesame (*Sesamum indicum* L.) plants exposed to water deficit. J Plant Pysiol 164, 1134–1143.

Kleber, H.-P., Schlee, D., Schöpp, W., 1997. Biochemisches Praktikum. Methoden für Studium, Praxis, Forschung. G. Fischer, Jena.

Kodra, E., Steinhaeuser, K., Ganguly, A., 2011. (2011), Persisting cold extremes under 21st-century warming scenarios. Geophys Res Lett, doi:10.1029/2011GL047103.

Koenker, R., 2006. Quantile Regression. R package version 4.02. http://www.r-project.org.

Kolattukudy, P., Croteau, R., Buckner, J., 1976. Biochemistry of plant waxes. In: Kolattukudy, P. (Ed.), Chemistry and Biochemistry of Natural Waxes. Elsevier, Amsterdam, pp. 290–347.

Kölling, C., 2007. Klimahüllen für 27 Waldbäume. AFZ - Der Wald 23, 1242–1245.

Kreyling, J., 2010. Winter climate change: a critical factor for temperate vegetation performance. Ecology 91, 1939–1948.

Kreyling, J., Bittner, T., Jaeschke, A., Jentsch, A., Steinbauer, M.J., Thiel, D., Beierkuhnlein, C., 2011a. Assisted colonization - a question of focal units and recipient localities. Restor Ecol 19, 433-440.

Kreyling, J., Henry, H.A.L., 2011. Vanishing winters in Germany: soil frost dynamics and snow cover trends, and ecological implications. Clim Res 46, 269–276.

Kreyling, J., Thiel, D., Nagy, L., Jentsch, A., Huber, G., Konnert, M., Beierkuhnlein, C., 2011b. Late frost sensitivity of juvenile *Fagus sylvatica* L. differs between southern Germany and Bulgaria and depends on preceding air temperature. Europ J Forest Res 131: 717-725.

Korner, C, Paulsen, J. 2004. A world-wide study of high altitude treeline temperatures. J Biogeogr 31: 713–732.

Kozlowski, T.T., Pallardy, S.G., 2002. Acclimation and adaptive responses of woody plants to environmental stresses. Bot Rev 68, 270–334.

Kuser, J.E., Ching, K.K., 1980. Provenance variation in phenology and cold hardiness of western hemlock seedlings. Forest Sci 26, 463–470.

Maffei, M., Badino, S., Bossi, S., 2004. Chemotaxonomic significance of leaf wax n-alkanes in the Pinales (Coniferales). Journal of Biological Research 1, 3–19.

Mancuso, S., 2000. Electrical resistance changes during exposure to low temperature measure chilling and freezing tolerance in olive tree (*Olea europaea* L.) plants. Plant Cell Environ 23, 291–299.

McKenney, D., Pedlar, J., O'Neill, G., 2009. Climate change and forest seed zones: Past trends, future prospects and challenges to ponder. Forest Chron 85, 258–266.

Moellering, E.R., Muthan, B., Benning, C., 2010. Freezing tolerance in plants requires lipid remodeling at the outer chloroplast membrane. Science 330, 226–228.

Morin, X., Ameglio, T., Ahas, R., Kurz-Besson, C., Lanta, V., Lebourgeois, F., Miglietta, F., Chuine, I., 2007. Variation in cold hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three European oak species. Tree Physiol 27, 817–825.

Nkongolo, K.K., Michael, P., Gratton, W.W., 2002. Identification and characterization of RAPD markers inferring genetic relationships among Pine species. Genome 45, 51–58.

Parmesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421, 37–42.

Pinheiro, J.C., Bates, D.M., 2004. Mixed-effects models in S and S-PLUS. Springer, New York.

Prior, S.A., Pritchard, S.G., Runion, G.B., Rogers, H.H., Mitchell, R.J., 1997. Influence of atmospheric CO_2 enrichment, soil N, and water stress on needle surface wax formation in *Pinus palustris* (Pinaceae). Am J Bot 84, 1070–1077.

R Development Core Team, 2010. [R: A Language and Environment for Statistical Computing. R version 2.11.1. R Foundation for Statistical Computing. ISBN 3-900051-07-0, URL http://www.R-project.org, Vienna, Austria.

Radler, F., Horn, D.H., 1965. Composition of grape cuticle wax. Aust J Chem 18, 1059-&.

Repo, T., Mononen, K., Alvila, L., Pakkanen, T.T., Hanninen, H., 2008. Cold acclimation of pedunculate oak (*Quercus robur* L.) at its northernmost distribution range. Environ Exp Bot 63, 59–70.

Saenz-Romero, C., Tapia-Olivares, B.L., 2008. Genetic variation in frost damage and seed zone delineation within an altitudinal transect of *Pinus devoniana* (*P. michoacana*) in Mexico. Silva Genet 57, 165–170.

Sakai, A., Weiser, C.J., 1973. Freezing resistance of trees in North America with reference to tree regions. Ecology 54, 118–126.

Schaberg, P.G., Hennon, P.E., D'Amore, D.V., Hawley, G.J., 2008a. Influence of simulated snow cover on the cold tolerance and freezing injury of yellow-cedar seedlings. Glob Change Biol 14, 1282–1293.

Schaberg, P.G., DeHayes, D.H., Hawley, G.J., Nijensohn, S.E., 2008b. Anthropogenic alterations of genetic diversity within tree populations: Implications for forest ecosystem resilience. Forest Ecol Manag 256, 855–862.

Schär, C, Vidale, PL, Luthi, D, Frei, C, Haberli, C, Liniger, MA, Appenzeller, C. 2004. The role of increasing temperature variability in European summer heatwaves. Nature 427: 332–336.

Shaw, M.R., Zavaleta, E.S., Chiariello, N.R., Cleland, E.E., Mooney, H.A., Field, C.B., 2002. Grassland responses to global environmental changes suppressed by elevated CO_2. Science 298, 1987–1990.

Shepherd, T., Griffiths, D.W., 2006. The effects of stress on plant cuticular waxes. New Phytol 171, 469–499.

Strimbeck, G.R., Kjellsen, T.D., Schaberg, P.G., Murakami, P.F., 2007. Cold in the common garden: comparative low-temperature tolerance of boreal and temperate conifer foliage. Trees-Struct Funct 21, 557–567.

Sutinen, M.L., Palta, J.P., Reich, P.B., 1992. Seasonal differences in freezing stress resistance of needles of *Pinus nigra* and *Pinus resinosa* - evaluation of the electrolyte leakage method. Tree Physiol 11, 241–254.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B. F. N., de Siqueira, M. F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A. S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T., Phillips, O.L., Williams, S.E., 2004. Extinction risk from climate change. Nature 427, 145–148.

Thomashow, M.F., 1999. Plant cold acclimation: Freezing tolerance genes and regulatory mechanisms. Ann Rev Plant Physiol Plant Molec Biol 50, 571–599.

Varelides, C., Brofas, G., Varelides, Y., 2001. Provenance variation in *Pinus nigra* at three sites in Northern Greece. Ann Forest Sci 58, 893–900.

Vavrus, S., Walsh, J.E., Chapman, W.L., Portis, D., 2006. The behavior of extreme cold air outbreaks under greenhouse warming. Int J Climatol 26, 1133–1147.

Visnjic, V.C., Dohrenbusch, A., 2004. Frost resistance and phenology of European beech provenances (*Fagus sylvatica* L.). Allg Forst Jgdztg 175, 101–108.

Watt, M.S., Ganley, R.J., Kriticos, D.J., Manning, L.K., 2011. *Dothistroma* needle blight and pitch canker: the current and future potential distribution of two important diseases of *Pinus* species. Can J Forest Res 41, 412–424.

Wiesenberg, G.L.B., Schmidt, M.W.I., Schwark, L., 2008. Plant and soil lipid modifications under elevated atmospheric CO2 conditions: I. Lipid distribution patterns. Org Geochem 39, 91–102.

Wiesenberg, G.L.B., Gocke, M., Kuzyakov, Y., 2010. Fast incorporation of root-derived lipids and fatty acids into soil - Evidence from a short term multiple (CO_2)-C-14 pulse labelling experiment. Org Geochem 41, 1049–1055.

Zobel, B.J., Wyk, G.v., Stahl, P., 1987. Growing Exotic Forests. Wiley, New York, N.Y.

6.6. Manuskript 6:

Late frost sensitivity of juvenile Fagus sylvatica L. differs between southern Germany and Bulgaria and depends on preceding air temperature

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Keywords

Frost sensitivity, Genetic diversity, European beech, Ecotypes, Global warming

Abstract

Fagus sylvatica, the dominant native forest tree species of Central Europe, is sensitive to late frost events. Advanced leaf flushing due to climate warming may lead to more frequent frost damage in the future. Here, we explore local adaptation to late frost events at both continental and regional scales and test how moderate climate warming (+1.5°C) affects late frost sensitivity.

Short-term leaf injury and height growth after a late frost event were quantified in a common garden experiment with two-year old *F. sylvatica* seedlings. The fully crossed three factorial design consisted of a late frost manipulation, a continuous warming manipulation and selected provenances (three provenances from western Bulgaria and three from southern Germany).

Late frost led to leaf injury and reduced height growth (-7%). Provenances differed in their late frost sensitivity at the regional scale and local adaptation was detected. At the larger scale, the Bulgarian provenances showed reduced height growth (-17%) while the German provenances did not exhibit growth reduction. The warming treatment prevented late frost damage while height growth declined by 19% in the reference temperature treatment. This surprising finding was attributed to advanced leaf maturity in the warming treatment.

The impact of late frost events on *F. sylvatica* in a warmer world will depend on timing. An event that damages leaves immediately after leaf flushing appears negligible a few days earlier or later, thereby complicating projections. Local adaptation to late frost is evident at a regional scale. Management strategies should aim at maximizing genetic diversity to adapt to climate change.

Introduction

Frost events in spring can damage plants and lead to strong economic impacts (Gu et al. 2008). *Fagus sylvatica*, the naturally dominant forest tree species of Central Europe over a wide range of environmental conditions (Leuschner et al. 2006), can tolerate very cold conditions over winter, but is sensitive to late frost events after leaf flushing (Dittmar et al. 2006; Ningre and Colin 2007). Such late frost events may even determine its northern and north-eastern range limit (Bolte et al. 2007). The earlier onset of the vegetation period and increased climatic variability due to climate warming (Walther et al. 2002) may lead to an increased risk of late frost damage (Gu et al. 2008; Kramer 1994; Norby et al. 2003; Rigby and Porporato 2008).

Due to its wide geographic distribution, *F. sylvatica* covers a broad range of climatic conditions (4 to 12°C mean annual temperature and 450 to 2000 mm mean annual precipitation according to Kölling 2007). Phenotypic plasticity and the adaptive potential of forest trees are determined by their genetic composition (Hosius et al. 2006, Schaberg et al. 2008). In Central Europe, *F. sylvatica* exhibits a high genetic diversity within populations (Konnert 1995; Müller-Starck et al. 1992; Vornam et al. 2004). This pattern changes on a continental scale, where stronger genetic differentiation between populations becomes evident (Comps et al. 1990; Magri et al. 2006). Allels involved in temperature-related adaptive differentiation of individuals are described for *F. sylvatica* (Jump et al. 2006). No comparable information exists yet concerning drought or frost stress (Bolte 2006). Nevertheless, provenance trials have a long history in forestry (e.g. Burger 1948; Krahl-Urban 1958) and generally demonstrate a differential performance between provenances from different geographic origins (e.g. von Wuehlisch et al. 1995). Provenances from distinct geographic locations further show specific tolerance against abiotic stressors such as drought (e.g. Nielsen and Jorgensen 2003; Peuke et al. 2006; Tognetti et al. 1995), ozone (Paludan-Müller et al. 1999), and minimum winter temperature (Visnjic and Dohrenbusch 2004). Here, in an experimental approach we focus on the response to frost events in late spring. Local

adaptation to late frost events has been reported between The Netherlands and Germany (Kramer 1994) and along a gradient of continentality from northern Germany to eastern Poland, although the differentiation there is small (1.6°C difference in LT50) (Czajkowski and Bolte 2006b). Local adaptation to late frost events appears to be a common feature in forest trees, as it is also reported for four *Larix* species in Russia (Eysteinsson et al. 2009), *Abies alba* in Croatia (Ivankovic et al. 2007) and *Tsuga heterophylla* in the Pacific Northwest of North America - here accompanied by a trade-off between spring frost tolerance and growth (Hannerz et al. 1999).

Delayed leaf flushing is a general adaptation against late frost events (Nielsen and Jorgensen 2003; von Wuehlisch et al. 1995). Leaf flushing in *F. sylvatica* is strongly linked to temperature degree sums of the late winter and spring (von Wuehlisch et al. 1995). Furthermore, provenances from the east and south-eastern part of the natural distribution range tend to flush earlier than provenances of the western part of the range with respect to temperature degree sums (von Wuehlisch et al. 1995).

Such differences in phenology within the species clearly matter for the response of temperate forests in times of climate change (Kramer et al. 2000). The interaction between warming and late frost sensitivity, however, has not yet been explicitly tested experimentally. The response to such interactions might differ considerably from single factor experiments (Norby and Luo 2004; Shaw et al. 2002). For instance, exposure to elevated CO_2 increased freezing sensitivity in multiple alpine species without affecting the phenology (Martin et al. 2010).

Here, six provenances of *F. sylvatica* from Bulgaria and southern Germany (Bavaria) were tested for their late frost sensitivity in a common garden experiment. We hypothesized that (1) late frost events damage leaves and reduce growth of *F. sylvatica* with (2) provenances from origins that are more prone to late frost events being more tolerant against late frost events (local adaptation). We furthermore expected that (3) temperature conditions prior to the late frost event alter late frost tolerance, with higher frost tolerance in colder mean conditions. Here, we focus on a comparison between the continental scale (Bulgaria versus southern Germany) and a regional scale (three distinct provenances from each country) in order to investigate scale dependencies of local adaptation. This may be a first step towards identifying provenances adapted to future climates, which is viewed as a promising adaptation strategy in forestry and nature conservation in the face of climate change (Kreyling et al. 2011; Nielsen and Jorgensen 2003).

Materials and Methods

This common garden experiment was established in Bayreuth, Germany (49°55'19" N, 11°34'55" E) in March 2010. The long-term mean annual temperature for the site is 8.2°C, whereas long-term mean annual precipitation is 724 mm. Between 2001 and 2009, late frost events in May with minimum near-surface air temperatures between -2°C and -6°C occurred on our field site in every year (data: Department of Micrometeorology, University of Bayreuth). These events typically occur around dawn after clear nights, minimum temperatures therefore do not last longer than 3 hours.

Three provenances from Bulgaria and three provenances from Germany were selected due to their silvicultural importance and their inclusion in ongoing long-term provenance trials (Konnert and Huber, unpublished data). Geographic origin and climatic information on the origins are provided in Figure 1 and Table 1.

Table 1: Origin of provenances used in the experiment and climatic information (MAT: Mean Annual Temperature; MAP: Mean Annual Precipitation; May min: Mean Minimum Temperature in May), all data for the period 1950 to 2000 from worldclim (Hijmans et al 2005).

provenance	country	latitude	longitude	altitude (m asl)	MAT (°C)	MAP (mm)	May min. (°C)
Gotze Delchev	Bulgaria	N 41°38'08"	E 23°35'08"	1600	11.4	696	3.9
Petrochan	Bulgaria	N 43°15'18'	E 23°14'20"	200	10.4	1004.1	9.1
Strumjani	Bulgaria	N 41°40'55"	E 23°00'42"	1450	13.9	676	4.3
Hengstberg	Germany	N 50°08'00"	E 12°11'00"	569	6.8	758	6
Elchingen	Germany	N 48°27'21"	E 10°03'48"	560	8.1	705	6.6
Weildorf	Germany	N 47°50'45"	E 12°52'56"	460	7.9	1325	7.3

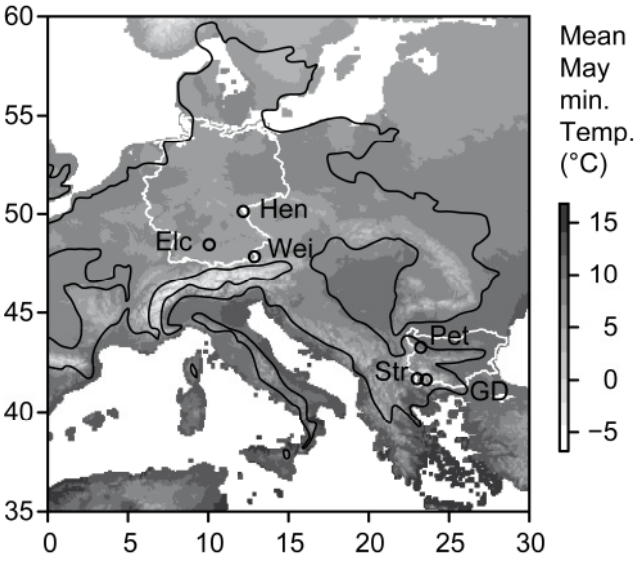

Figure 1: Location of the target provenances within the distribution of *Fagus sylvatica* (black lines; simplified from EUFORGEN (2009) to mainly display outer distribution limits) in Germany and Bulgaria (white outlines). Elc: Elchingen, Hen: Hengstberg, Wei: Weildorf, Pet: Petrochan, Str: Strumjani, GD: Gotze Delchev. Grey scales display the minimum temperature in May in 5' spatial resolution (Hijmans et al. 2005).

The target provenances were cultivated at the *Bavarian Institute for Forest Seeding and Planting (ASP)* in Teisendorf, Germany from April 2009 to March 2010. The seedlings were then transported to Bayreuth and individually planted into 12-litre plastic pots filled with sandy silt (pH 6.7, total C 1.58%, total N 0.13%, CEC 131 mmolc/kg). Selection of plants occurred randomly for each provenance from all plants alive at planting date. Mean plant size at the start of the experiment was 12.5 cm ± 5.0 cm SD. The irrigation simulated the local daily 30-year average precipitation applied twice a week with collected rain water (soil moisture data is provided in Figure 2).

The potted individuals were exposed to the fully crossed threefold factorial combination of (1) a late frost manipulation (late frost and control) and (2) a continuous warming manipulation (warming and reference) and (3) a provenance treatment (three Bulgarian and three German provenances). The late frost manipulation took place in the night from 26th to 27th of May 2010. Based on local climate data, a late frost event of -5°C for three hours was simulated by gradual cooling inside a cooler truck. From April to October 2010, the plants were exposed to the warming treatment. The two levels of the warming treatment were replicated three times, resulting in six experimental units. Each experimental unit was covered by a single large rain-out shelter (11 m by 7 m, 3.8 m in height) constructed of a steel frame (GlasMetall Riemer GmbH) and covered with a transparent polyethylene sheet (0.2 mm, SPR5, Hermann Meyer GmbH). The edge of the rain-out shelters was at a height of 80 cm

and the sheets permitted nearly 90% penetration of photosynthetically active radiation. Shading nets reduced radiation by another 30 % as *F. sylvatica* regeneration usually occurs sheltered below an open tree canopy. The warming manipulation took place both passively (wind-shelters reducing wind speed by 70% and black floor-covers versus white floor covers) and actively (IR-radiation with approximately 30 W per m²), which increased the average air temperature at plant height by 1.6°C (Figure 2). The late frost manipulation and the provenance treatment were nested within each experimental unit. Each provenance was further replicated with three plants per late frost treatment and experimental unit (nested replicates), resulting in 9 plants per factorial combination of the three-factorial design and 216 plants overall. The available plants were assigned randomly to the experimental units.

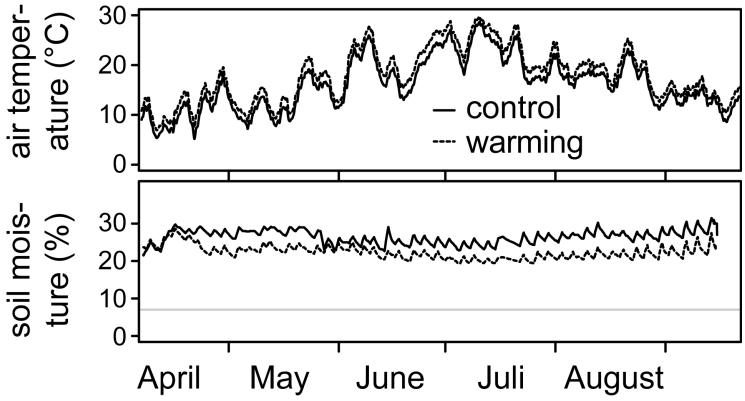

Figure 2: Air temperature and soil moisture over the course of the experiment. Permanent wilting point (pF = 4.2) is indicated by the grey line in the lower panel.

Short-term response to the late frost manipulation was determined by visual estimates by assigning the average percentage of chlorotic and necrotic leaf area per plant to five classes (0-5%, 5-25%, 25-50%, 50-75%, 75-100%). This assessment took place five days after the late frost manipulation. For each individual, midpoints of the five classes were used for further statistical analyses. Long term effects of the late frost manipulation were measured by total plant height at the end of the growing season in October. Leaf phenology was documented weekly based on the BBCH code (Meier 2001). Here, we focus on the end of leaf onset (stage 19 of this code) which is the date when the leaves are fully developed to their species-specific size and color. This stage was selected as it was reached around the date of the late frost manipulation and is presumably the end of the most sensitive phenological phase against frost. Furthermore, leaf senescence in autumn was recorded.

Mean minimum temperatures in May for the period 1950 to 2000 for each geographic origin of the provenances retrieved from worldclim (Hijmans et al. 2005) were used as indicators for late frost occurrences (Table 1). Although these values exceed absolute minimum temperatures considerably due to averaging (for our experimental site mean minimum temperature in May based on worldclim is 7.5°C while absolute minimum temperatures between 2001 and 2009 ranged between -2 and -6°C), we assume that the relative differences between geographic origins are adequately captures. We use these surrogates because climate stations are not available in reasonable vicinity to our geographic origins (> 10 km). An ordinary least squares regression between this indicator and late frost sensitivity, expressed as the difference in leaf damage or height between control and frost-manipulated plants, was applied for each warming treatment in order to detect local adaptation to late frost events.

Analysis of variance (ANOVA) combined with linear mixed effect models were applied to test for the main and interactive effects of the three factors: provenance, late frost, and warming. Including the experimental unit as a random factor accounted for the split-plot design and the nested replicates (Pinheiro and Bates 2004). The same models were run a second time with provenance used as an additional random factor and country (Bulgaria and Germany) used as a fixed factor to determine if provenance effects were still visible on the larger geographic scale. Data were log transformed to improve the homogeneity of variances and the normality of residuals prior to analysis if necessary (Faraway 2006). In case of significant interaction effects, post-hoc comparisons (Tukey's test) were run according to (Hothorn et al. 2008). All statistical analyses were conducted with the software R 2.11.1 (R Development Core Team 2010) and the additional packages "nlme", "multcomp".

Results

Late Frost Events Affect *Fagus Sylvatica*

The late frost manipulation caused leaf damage which on average affected 14.5% of the leaf area in the late frost manipulated plants five days after the manipulation, while no leaf injury was observed in the control plants ($F = 122.2$; $p < 0.001$, see Table 2 for all ANOVA results). The height of the plants at the end of the growing season was reduced by 1.4 cm on average due to the late frost manipulation, equating to a reduction of 7% ($F = 4.4$; $p = 0.038$). The phenology of leaf onset was not affected by the late frost event. Leaf senescence, however,

was brought forward by 1.6 days for those plants that experienced the late frost manipulation ($F = 10.8$; $p = 0.001$).

Local Adaptation

Provenances differed in their short-term late frost sensitivity, as expressed by leaf damage 5 days after the manipulation (interaction between provenance and late frost manipulation: $F = 2.5$; $p = 0.025$; Figure 3A). The German provenances exhibited more frost damage than the Bulgarian ones (interaction between country and late frost: $F = 7.9$; $p = 0.005$; horizontal lines in Figure 3). The German provenance from Hengstberg, however, showed a similarly low level of leaf damage to the Bulgarian ones.

The provenances also differed in height increment ($F = 3.8$; $p = 0.001$; Figure 3B). Here, the provenance from Petrochan performed best under control conditions, but also exhibited the strongest growth reduction after the late frost manipulation. The provenance from Elchingen, on the other hand, even showed an increased height growth after late frost and performed best under this treatment. The late frost manipulation only affected height growth negatively in the Bulgarian provenances, which experienced a reduction of 17%, while the German provenances showed no late frost effect at all (interaction between country and late frost: $F = 6.0$; $p = 0.015$; horizontal lines in Figure 3B).

Provenances from origins that are more prone to late frost events (Mean mimimum temperature in May, Table 2) tended to be more tolerant against late frost events, as indicated by relatively strong correlations between late frost sensitivity and minimum temperature in May at the geographic origin of the provenances (Figure 4). No such correlation, however, was detected for the height differential in the warming treatment.

Table 2: ANOVA results of the applied mixed models. Upper panel: regional scale (fixed factors: provenance, late frost, warming; random factor: experimental unit). Lower panel: continental scale (fixed factors: country, late frost, warming; random factors: experimental unit, provenance). Significant effects are set off bold.

parameter		leaf injury		height		leaf onset		leaf senescence	
	denDF*	F	p	F	p	F	p	F	p
provenance	216	**2.4**	**0.029**	**3.8**	**0.001**	**3.8**	**0.004**	0.6	0.735
late frost	216	**122.2**	**<0.001**	**4.4**	**0.038**	3.0	0.107	**10.8**	**0.001**
warming	216	**13.7**	**<0.001**	0.0	0.875	2.5	0.276	**6.2**	**0.014**
provenance:late frost	216	**2.5**	**0.025**	1.8	0.093	1.7	0.231	1.3	0.267
provenance:warming	216	0.4	0.869	1.1	0.387	2.9	0.065	0.8	0.549
late frost:warming	216	**13.5**	**<0.001**	**12.7**	**<0.001**	0.1	0.987	3.8	0.050
provenance:late frost:warming	216	0.5	0.789	1.8	0.099	1.2	0.772	0.9	0.456
country	14	**8.5**	**0.012**	2.7	0.124	0.4	0.643	0.0	0.897
late frost	188	**97.2**	**<0.001**	2.7	0.100	2.7	0.125	**10.6**	**0.001**
warming	188	**11.5**	**0.001**	0.1	0.849	**6.9**	**0.013**	**6.1**	**0.014**
country:late frost	188	**7.9**	**0.005**	**6.0**	**0.015**	1.1	0.379	0.0	0.897
country:warming	188	0.1	0.863	0.6	0.447	1.3	0.371	0.2	0.696
late frost:warming	188	**10.8**	**0.001**	**12.5**	**<0.001**	0.3	0.754	3.8	0.052
country:late frost:warming	188	0.1	0.813	3.9	0.051	1.6	0.270	0.2	0.696

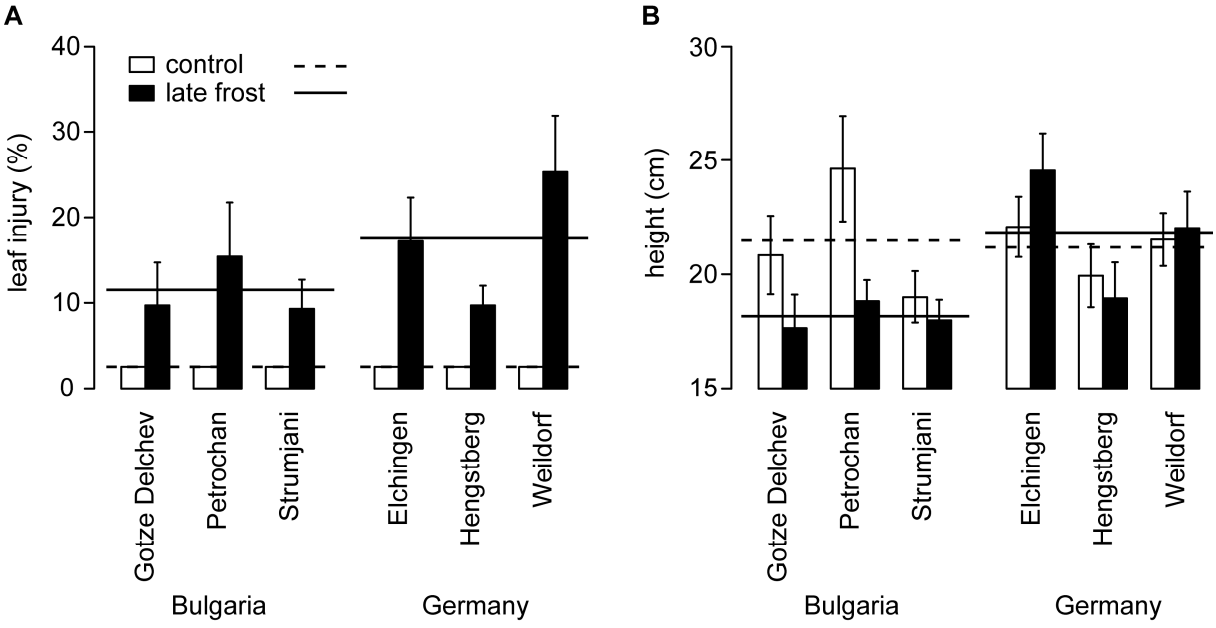

Figure 3: (A) leaf damage five days after the late frost manipulation, and (B) height in October were affected by the late frost event which took place on May 26th. ANOVA-results are provided in Table 2. Mean values and standard errors are shown over 18 individuals per bar. Horizontal lines indicate the

mean response per country. Note that midpoints of the five classes are used for calculations of leaf damage, which leads to mean values above zero for the controls.

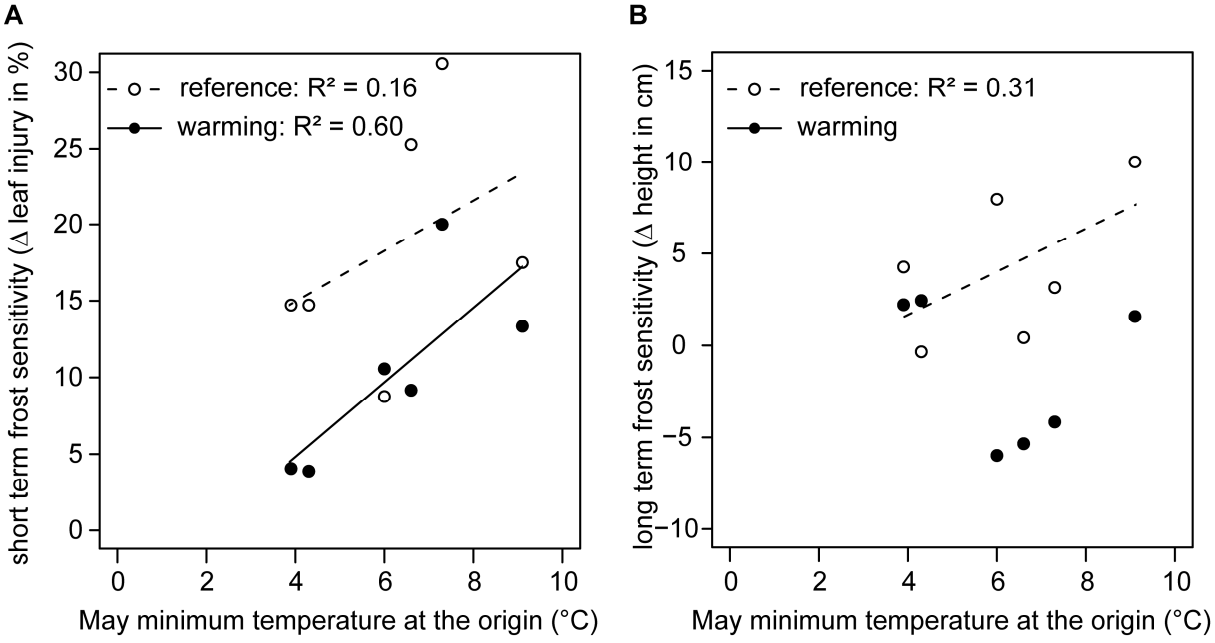

Figure 4: Local adaptation of frost sensitivity expressed by (A) the difference in leaf damage between reference and frost manipulation and the minimum temperature in May at the origin five days after the late frost manipulation which took place on May 26th, and (B) the difference in height by October between the reference and the frost manipulation and the minimum temperature in May at the origin. Linear regressions are shown if significant ($p<0.1$ due to small sample number).

Preceeding Temperature Alters Late Frost Sensitivity

Warming reduced the late frost tolerance. Leaf injury was significantly higher in plants, which experienced colder temperatures throughout the experiment in comparison to those that were warmed continuously (Figure 5A). Height growth was reduced by 19% due to late frost in the absence of continuous warming, while no such difference was found with warming (Figure 5B).

Phenology, which was unaffected by the late frost manipulation and which did not differ between the countries (Table 2), was advanced by 2 days in the warming treatment ($F = 6.9$; $p = 0.013$), again without significant interactions with the late frost manipulation or the country of origin.

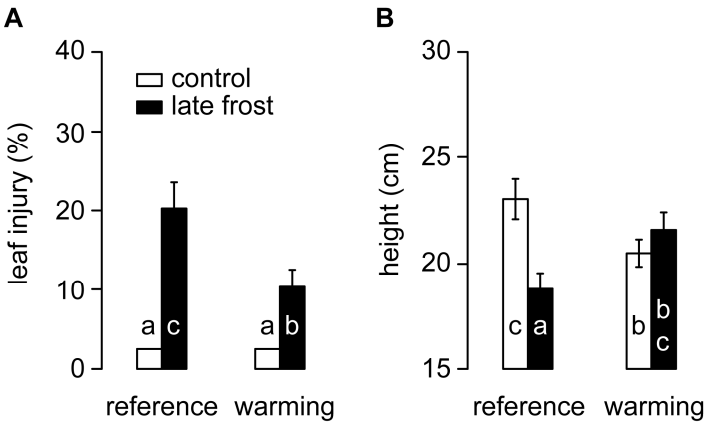

Figure 5: Preceding air temperature effects on (A) leaf damage and (B) height growth in response to the late frost manipulation. Lower case letters indicate homogeneous groups as detected by Tukey-post hoc comparisons. No significant interactions were detected with country or provenance (threefold interactions in Table 2). Mean values and standard errors are shown over 54 individuals per bar.

Discussion

Late Frost Events Affect *Fagus sylvatica*

Field observations (Gu et al. 2008; Norby et al. 2003) and model results (Kramer 1994; Rigby and Porporato 2008) indicate that an earlier onset of the vegetation period due to climate change may lead to an increased risk of late frost damage despite the general trend in air warming. Here, we found considerable short-term frost damage to the foliage, while height growth was reduced moderately (-7%). The strong short-term damage was followed by moderate reductions in the height increment. The fact that latent buds were activated may be one explanation for this effect (Awaya et al. 2009). Our findings emphasize once more that *F. sylvatica* is sensitive to late frost events after leaf flushing (Dittmar et al. 2006; Ningre and Colin 2007). Hence, frost events of minimal duration can impede the general trend of increased productivity due to an elongation of the growing season. Furthermore, the results imply that frost events and other winter processes are important drivers of temperate vegetation performance even, or especially, in a warming world (Inouye 2000; Kreyling 2010).

We used juvenile trees in their second year of development in this experiment. Generally, it can be expected that seedlings are more sensitive against late frost events than older trees (Bolte and Ibisch 2007; Ningre and Colin 2007). Similar differences in responsiveness between juvenile and adult individuals are described for ozone stress and attributed to higher stomatal conductance in juvenile trees (Herbinger et al. 2005; Nunn et al. 2005). However, the

juvenile stage is of high importance for the natural regeneration of forest stands. Moreover, the high selective pressure of single extreme events such as frost or drought can determine the genetic composition of future stands.

Local Adaptation

The selected provenances differed in their height growth and late frost sensitivity (Figure 3). Late frost sensitivity furthermore correlated with the minimum temperatures for May at the geographic origins of the species (Figure 4), thereby indicating the inheritance of local adaptation to late frost events, which was exhibited at the regional level rather than the continental level. This corresponds well with the genetic diversity of *F. sylvatica* at the population level (Konnert 1995; Müller-Starck et al. 1992; Vornam et al. 2004). Observations along a moisture gradient from Germany to Poland suggest, for instance, that drought tolerance increases with drier origins of provenances. However, all the provenances tested there included highly drought-tolerant individuals (Czajkowski and Bolte 2006a), a finding that emphasizes the importance of local genetic diversity. Genetic investigations (isozyme and neutral microsatellite markers) indicate a high genetic diversity within beech populations from Bulgaria (Cremer et al. 2009), confirming the well known "hot spots theory" (Petit et al. 2003) within the Balkan region. However, no explicit genetic differentiation was found between the German and Bulgarian beech provenances used in our experiment (Cremer et al. 2009).

Delayed leaf flushing has been suggested to be an important adaptation against frost in general (Visnjic and Dohrenbusch 2004) and late frost events in particular (Nielsen and Jorgensen 2003; von Wuehlisch et al. 1995). In our experiment, however, phenology did not differ between the Bulgarian and the German provenances while late frost sensitivity did. Based on a broader climatic gradient covered, provenances of *F. sylvatica* from both regions have been reported to have especially high resistance against winter frost, accompanied by late leaf flushing (Visnjic and Dohrenbusch 2004). We can only speculate on further physiological reasons for the differences in late frost sensitivity. In grasses, for instance, leaf proteins are reported to accumulate differentially during cold acclimation between genotypes with distinct levels of frost tolerance (Kosmala et al. 2009). Likewise, exposure to elevated CO_2 increased freezing sensitivity in multiple alpine species without affecting the phenology (Martin et al. 2010).

The climate has been a major driver of community compositions in the past and will continue to be in the future (Willis et al. 2010). Assisted colonization has therefore been discussed as a means to protect rare and endangered species (Hoegh-Guldberg et al. 2008). The functioning of ecosystems, however, might be another pressing need in times of fast climate change (Willis et al. 2010). The assisted colonization of pre-adapted ecotypes of key species might contribute to this goal without the need to introduce exotic species with unknown risks (Kreyling et al. 2011). Our results suggest that provenances of a key forest tree species in Central Europe differ in their late frost sensitivity. Considering this one factor, the correlations between home site climate and late frost sensitivity (Figure 4) even suggest that the selection of provenances could be possible based on the current climatic conditions within the species ranges (Nielsen and Jorgensen 2003). However, our finding that the provenance with the best performance under control conditions suffered the most by the late frost event (Figure 3B) while another provenance with mediocre growth in the control even increased its growth after the late frost event indicates that provenance trials under control conditions may be misleading under changing climatic mean and extreme conditions. The multitude of possible climatic variables to be selected and uncertainties concerning future climates imply that the search for best-adapted provenances may not be the best strategy. Instead, management actions, which promote genetic diversity itself would appear to be more preferable as genetic diversity enables organisms to continue to adapt and evolve to new circumstances within one or several generation cycles (Hosius et al. 2006; Jump et al. 2006; Kramer et al. 2010; Mace and Purvis 2008; Schaberg et al. 2008).

Our results are based on 9 plants per factorial combination. Results of the late frost effects therefore comprise 18 plants per provenance and treatment. Based on these numbers, the comparably low within provenance variance and the significance pattern of the analyses, we are confident that reliable results have been obtained. The regression analysis and the future discussion, however, would definitely benefit from an increased number of provenances from a wider range of origins such as southern Italy or France and Spain which are known to be more sensitive to frost damage (Visnjic and Dohrenbusch 2004).

Preceeding Temperature Alters Late Frost Sensitivity

In our experiment warming advanced the phenological leaf onset by two days irrespective of geographic origin. A similar phenotypic plasticity has been reported for *F. sylvatica* along a regional altitudinal gradient (Vitasse et al. 2010). Against our expectations, this advance in

phenology did not increase, but reduced frost damage after the late frost manipulation (Figure 5). As far as our target species *F. sylvatica* is concerned, there appears to be only a short window of very high frost sensitivity directly after leaf flushing. As soon as the leaves mature, their frost tolerance appears to increase again. This result implicates that the timing of frost events relative to phenological development is crucial and that this is not a question of a one-sided threshold (i.e. bud burst). Clearly, leaf maturity should also be taken into account. This complicates the projection of future late frost damage, as phenology and late frost incidents need to be predicted with a very high (daily) temporal resolution. Clearly, experiments on interacting climatic drivers are urgently needed, as the response to such interactions might differ considerably from single factor experiments (Norby and Luo 2004; Shaw et al. 2002).

Conclusions

Late frost events affect the performance of *F. sylvatica*. It has been suggested that the impact of such events may even increase in a warmer world, as they might hit vegetation that is further developed. Our results, however, suggest that the timing of late frost events even after bud burst is crucial, as leaves appear to increase rapidly in frost tolerance after leaf flushing. Therefore, there is a need for reliable projections of late frost risks in the future.

F. sylvatica exhibited local adaptation in late frost sensitivity not only at the continental scale, but already at a regional scale. The high within-species variability should be maintained, or even increased, for adaption to climate change.

Ultimately, tree species responses should be regarded in the context of populations under competitive pressure. The advantage of common garden experiments is that they can detect the spectrum of possible species-specific responses. Nevertheless, there is a need to test the obtained results in communities where the competitive balance might amplify or buffer responses.

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References

Awaya Y, Tanaka K, Kodani E *et al* (2009) Responses of a beech (*Fagus crenata* Blume) stand to late spring frost damage in Morioka, Japan. Forest Ecol Manag 257:2359–2369

Bolte A (2006) Zur Zukunft der Buche in Mitteleuropa. AFZ - Der Wald 20:1077–1078

Bolte A, Ibisch PL (2007) Neun Thesen zu Klimawandel, Waldbau und Naturschutz. AFZ - Der Wald 11:572–576

Bolte A, Czajkowski T, Kompa T (2007) The north-eastern distribution range of European beech - a review. Forestry 80:413–429

Burger H (1948) Einfluss der Herkunft des Samens auf die Eigenschaften forstlicher Holzgewächse. VI Mitteilungen: Die Buche. Mitteilungen Schweiz Anst Forstl Versuchsw 25:287–326

Comps B, Thiebaut B, Paule L *et al* (1990) Allozymic variability in beechwoods (*Fagus sylvatica* L) over Central-Europe - spatial differentiation among and within populations. Heredity 65:407–417

Cremer E, Petkova G, Huber G, Konnert M (2009) Transfer of German beech nuts to Bulgaria and vice versa. In: Zhelev P (ed) Abstracts of the International Scientific Conference "Balkans - hot spots of ancient and present genetic diversity", Sofia, Bulgaria

Czajkowski T, Bolte A (2006a) Unterschiedliche Reaktion deutscher und polnischer Herkünfte der Buche (*Fagus sylvatica* L.) auf Trockenheit. Allgemeine Forst- und Jagdzeitschrift 177:30–40

Czajkowski T, Bolte A (2006b) Frosttoleranz deutscher und polnischer Herkünfte der Buche (*Fagus sylvatica* L.) und ihre Beeinflussung durch Trockenheit. Archiv für Forstwesen und Landschaftsökologie 40:119–126

Dittmar C, Fricke W, Elling W (2006) Impact of late frost events on radial growth of common beech (*Fagus sylvatica* L.) in Southern Germany. Europ J Forest Res 125:249–259

EUFORGEN (2009) Distribution map of Beech (*Fagus sylvatica*). www.euforgen.org

Eysteinsson T, Karlman L, Fries A *et al* (2009) Variation in spring and autumn frost tolerance among provenances of Russian larches (*Larix* Mill.). Scand J Forest Res 24:100–110

Faraway JJ (2006) Extending the Linear Model with R - Generalized Linear, Mixed Effects and Nonparametric Regression Models. Chapman & Hall/CRC, Baco Raton

Gu L, Hanson PJ, Post WM *et al* (2008) The 2007 Eastern US spring freeze: increased cold damage in a warming world? Bioscience 58:253–262

Hannerz M, Aitken SN, King JN *et al* (1999) Effects of genetic selection for growth on frost hardiness in western hemlock. Can J Forest Res 29:509–516

Herbinger K, Then C, Low M *et al* (2005) Tree age dependence and within-canopy variation of leaf gas exchange and antioxidative defence in *Fagus sylvatica* under experimental free-air ozone exposure. Environment Pollut 137:476–482

Hijmans RJ, Cameron SE, Parra JL *et al* (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25:1965–1978

Hoegh-Guldberg O, Hughes L, McIntyre S *et al* (2008) Assisted colonization and rapid climate change. Science 321:345–346

Hosius B, Leinemann L, Konnert M *et al* (2006) Genetic aspects of forestry in the central Europe. Europ J Forest Res 125:407–417

Hothorn T, Bretz F, Westfall P (2008) Simultaneous Inference in General Parametric Models. Biometrical J 50:346–363

Inouye DW (2000) The ecological and evolutionary significance of frost in the context of climate change. Ecol Lett 3:457–463

Ivankovic M, Marjanovic H, Franjic J *et al* (2007) Variability of Silver fir (*Abies alba* Mill.) provenances in the number of lateral buds on terminal sprout and damage by the late frost. Period Biol 109:55–59

Jump AS, Hunt JM, Martinez-Izquierdo JA *et al* (2006) Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. Molec Ecol 15:3469–3480

Kölling C (2007) Klimahüllen für 27 Waldbäume. AFZ - Der Wald 23:1242–1245

Konnert M (1995) Investigations on the genetic variation of beech (*Fagus sylvatica* L) in Bavaria. Silva Genet 44:346–351

Kosmala A, Bocian A, Rapacz M *et al* (2009) Identification of leaf proteins differentially accumulated during cold acclimation between *Festuca pratensis* plants with distinct levels of frost tolerance. J Exp Bot 60:3595–3609

Krahl-Urban J (1958) Vorläufige Ergebnisse der Buchenprovenienzversuche. Allgemeine Forst- und Jagdzeitschrift 129:242–251

Kramer K (1994) A modeling analysis of the effects of climatic warming on the probability of spring frost damage to tree species in the Netherlands and Germany. Plant Cell Environ 17:367–377

Kramer K, Leinonen I, Loustau D (2000) The importance of phenology for the evaluation of impact of climate change on growth of boreal, temperate and Mediterranean forests ecosystems: an overview. Int J Biometeorol 44:67–75

Kramer K, Degen B, Buschbom J *et al* (2010) Modelling exploration of the future of European beech (*Fagus sylvatica* L.) under climate change-Range, abundance, genetic diversity and adaptive response. Forest Ecol Manag 259:2213–2222

Kreyling J (2010) Winter climate change: a critical factor for temperate vegetation performance. Ecology 91:1939–1948

Kreyling J, Bittner T, Jaeschke A *et al* (2011) Assisted colonization - a question of focal units and recipient localities. Restor Ecol 19: 433-440.

Leuschner C, Meier IC, Hertel D (2006) On the niche breadth of *Fagus sylvatica*. Ann. For. Sci 63:355–368

Mace GM, Purvis A (2008) Evolutionary biology and practical conservation: bridging a widening gap. Molec Ecol 17:9–19

Magri D, Vendramin GG, Comps B *et al* (2006) A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. New Phytol 171:199–221

Martin M, Gavazov K, Koerner C *et al* (2010) Reduced early growing season freezing resistance in alpine treeline plants under elevated atmospheric CO_2. Glob Change Biol 16:1057–1070

Meier, U (ed) (2001) Growth stages of mono-and dicotyledonous plants. BBCH Monograph, URL: http://www.jki.bund.de/de/startseite/veroeffentlichungen/bbch-codes.html

Müller-Starck G, Baradat P, Bergman F (1992) Genetic variation within European tree species. New Forests 6:23–47

Nielsen CN, Jorgensen FV (2003) Phenology and diameter increment in seedlings of European beech (*Fagus sylvatica* L.) as affected by different soil water contents: variation between and within provenances. Forest Ecol Manag 174:233–249

Ningre F, Colin F (2007) Frost damage on the terminal shoot as a risk factor of fork incidence on common beech (*Fagus sylvatica* L.). Ann Forest Sci 64:79–86

Norby RJ, Luo YQ (2004) Evaluating ecosystem responses to rising atmospheric CO_2 and global warming in a multi-factor world. New Phytol 162:281–293

Norby RJ, Hartz-Rubin JS, Verbrugge MJ (2003) Phenological responses in maple to experimental atmospheric warming and CO_2 enrichment. Glob Change Biol 9:1792–1801

Nunn AJ, Kozovits AR, Reiter IM *et al* (2005) Comparison of ozone uptake and sensitivity between a phytotron study with young beech and a field experiment with adult beech (*Fagus sylvatica*). Environmental Pollution 137:494–506

Paludan-Müller G, Saxe H, Leverenz JW (1999) Responses to ozone in 12 provenances of European beech (*Fagus sylvatica*): genotypic variation and chamber effects on photosynthesis and dry-matter partitioning. New Phytol 144:261–273

Petit RJ, Aguinagalde I, Beaulieu JL de *et al* (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. Science 300:1563–1565

Peuke AD, Gessler A, Rennenberg H (2006) The effect of drought on C and N stable isotopes in different fractions of leaves, stems and roots of sensitive and tolerant beech ecotypes. Plant Cell Environ 29:823–835

Pinheiro JC, Bates DM (2004) Mixed-effects models in S and S-PLUS. Springer, New York

R Development Core Team (2010) [R: A Language and Environment for Statistical Computing. R version 2.11.1. R Foundation for Statistical Computing. ISBN 3-900051-07-0, URL http://www.R-project.org, Vienna, Austria

Rigby JR, Porporato A (2008) Spring frost risk in a changing climate. Geophys Res Lett 35:L12703

Schaberg PG, DeHayes DH, Hawley GJ *et al* (2008) Anthropogenic alterations of genetic diversity within tree populations: Implications for forest ecosystem resilience. Forest Ecol Manag 256:855–862

Shaw MR, Zavaleta ES, Chiariello NR *et al* (2002) Grassland responses to global environmental changes suppressed by elevated CO_2. Science 298:1987–1990

Tognetti R, Johnson JD, Michelozzi M (1995) The response of European beech (*Fagus sylvatica* L.) seedlings from 2 Italian populations to drought and recovery. Trees-Struct Funct 9:348–354

Visnjic VC, Dohrenbusch A (2004) Frost resistance and phenology of European beech provenances (*Fagus sylvatica* L.). Allg Forst Jgdztg 175:101–108

Vitasse Y, Bresson CC, Kremer A *et al* (2010) Quantifying phenological plasticity to temperature in two temperate tree species. Funct Ecol 24:1211–1218

Vornam B, Decarli N, Gailing O (2004) Spatial distribution of genetic variation in a natural beech stand (*Fagus sylvatica* L.) based on microsatellite markers. Conserv Genet 5:561–570

Walther GR, Post E, Convey P *et al* (2002) Ecological responses to recent climate change. Nature 416:389–395

Willis KJ, Bailey RM, Bhagwat SA *et al* (2010) Biodiversity baselines, thresholds and resilience: testing predictions and assumptions using palaeoecological data. Trends Ecol Evol 25:583–591

Wuehlisch G von, Krusche D, Muhs HJ (1995) Variation in temperature sum requirement for flushing of beech provenances. Silva Genet 44:343–346

6.7. Manuskript 7:

Ecotypic differentiation and past climatic experience influence the response to late spring frost in four common grass species in Central Europe

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Local adaptation, provenance trial, cold tolerance, EVENT experiment, climate change, within-species diversity, intra-specific diversity

Abstract

Late spring frost events can affect vegetation. The response of grassland species, however, is generally unknown. We explore the late-frost sensitivity of four common European grass species and investigate whether these species exhibit local adaptations to late frost on a continental scale and whether past climatic experience influences late frost sensitivity.

Ecotypes of *Arrhenatherum elatius*, *Alopecurus pratensis*, *Festuca pratensis*, and *Holcus lanatus* from Spain, Italy, Bulgaria, Hungary, Sweden, and Germany were exposed to late frost after drought and warming manipulations in the preceding growing season in a common garden experiment.

Late frost reduced the productivity of the grasses on average by 20%. Ecotypes differed in their late-frost sensitivity in three of the four species and local adaptations to late frost were identified. Previous exposure to drought and warming caused differences in late-frost sensitivity in some cases.

The impact of late frost events may increase in a warmer world due to an earlier onset of growing and no change in timing of late frost events. The history of climatic exposure can

alter the performance of plants, possibly through epigenetic mechanisms. Based on the complex response pattern observed, a maximization of genetic diversity is proposed as a promising adaptation strategy against climate change.

Introduction

Frost events in the spring can damage plants, sometimes causing strong economic impacts (Gu et al. 2008). In addition to agriculturally important crops (Cromey et al. 1998), seed production of the dominant grass species of managed semi-natural grasslands may also be reduced (Hare 1995, Niemelainen 1991). The implications for biomass production, however, have not yet been studied in detail. Generally speaking, grasses have the ability to acclimate to frost through osmoregulation (Liu and Osborne 2008) and photosynthetic acclimation (Rapacz et al. 2004). Molecular mechanisms underlying winter frost tolerance in perennial grasses are reasonably well understood (Sandve et al. 2011). Dehardening, i.e., the loss of frost hardiness that protected the plants over winter, occurs within hours (Rapacz et al. 2000) leaving the plants vulnerable to short-term frost events during the growing season. An earlier onset of the growing season due to climate change may lead to an increased risk of late frost damage in spite of a general air warming trend, as the timing of late frost events is not changing (Gu et al. 2008, Rigby and Porporato 2008, Inouye 2008, Woldendorp et al. 2008). It has furthermore been predicted that plants will fail to optimize the trade-off between increasing their growing season by an earlier start of growing in a warmer world and the decreasing, yet still existent, risk of frost damage (Bennie et al. 2010).

Plants grown under generally warmer conditions may lack adaptations to frost (Eccel et al. 2009). However, plants can cope with different environmental stressors by similar responses at the cellular and molecular level when these stressors trigger similar signal chains. Drought and frost, for instance, lead to the accumulation of low-molecular-weight osmolytes that prevent cellular dehydration (Beck et al. 2007). Epigenetic changes and accumulation of signaling proteins or transcription factors can facilitate fast protective responses to recurrent or similar stressors (Bruce et al. 2007, Bossdorf et al. 2008). Gene expressions may persistently change due to stress and such epigenetic modifications can even be inherited through mitosis and meiosis (Aubin-Horth and Renn 2009, Bird 2007, Goh et al. 2003, Verhoeven et al. 2010). Long-term experiments on growth performance, however, are largely missing as most work on epigenetics up to now has been related to molecular mechanisms.

Due to their wide geographic distribution, perennial temperate grass species occur under a broad range of climatic conditions and are known to include climatically-adapted ecotypes (Macel et al. 2007, Ofir and Kigel 2010, Beierkuhnlein et al. 2011), i.e., distinct genotypes that are adapted to a local environment (Hufford and Mazer 2003). The identification of ecotypes that will be adapted to future climates is viewed as a promising adaptation strategy in agriculture and nature conservation in the face of climate change (Humphreys et al. 2006, Kreyling et al. 2011). Here, the ecotypes of four native grass species (*Arrhenatherum elatius*, *Festuca pratensis*, *Holcus lanatus*, *Alopecurus pratensis*) from contrasting climates across Europe were tested for their tolerance of late frost events in a common garden experiment. The target species are wide-spread across Europe and important for agriculture in permanent grasslands (Beierkuhnlein et al. 2011). These temperate grass species display large-scale genetic patterns in spite of being favored in their distribution by anthropogenic land use over centuries (Michalski et al. 2010).

Here, we tested for differences in late-spring frost sensitivity between ecotypes of four common grass species from origins all over Europe under different climate change scenarios (warming, summer drought) in a common garden experiment. We hypothesized that (1) late-spring frost events reduce both photosynthetic capacity and biomass production in temperate European grass species. We furthermore expected that (2) tolerance to late frost events increases with the occurrence of such events at the origin of the ecotypes (local adaptation) and that (3) past climatic experiences alter late frost tolerance, with the highest frost tolerance exhibited by individuals formerly grown in colder conditions and for individuals that experienced summer drought in the preceding growing season.

Materials and Methods

This common garden experiment was established in Bayreuth, Germany (49°55’19” N, 11°34’55” E) in April 2009. The long-term mean annual temperature for the site is 8.2°C, whereas the long-term mean annual precipitation is 724 mm. Between 2001 and 2009, late frost events in May with minimum near-surface air temperatures between -2°C and -6°C occurred on our field site every year (data: Department of Micrometeorology, University of Bayreuth).

The target species *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl, *Festuca pratensis* Huds. s.l., *Holcus lanatus* L and *Alopecurus pratensis* L. play major roles in extensively used hay meadows of Central Europe. *A. elatius* is mainly found on moist to moderately dry nutrient-rich soils, *F. pratensis* grows predominantly on fresh soils rich in

nutrients and humus, *H. lanatus* occurs mainly on wet and boggy soils, and *A. pratensis* is a common grass in moist and nutrient-rich soils (Oberdorfer 2001). As perennial clonal grasses, all four species share comparable life cycles and strategies for resource allocation.

The basic experimental design except for the late frost manipulation is described in detail by Beierkuhnlein *et al.* (2011). In addition to local ecotypes from Germany (DE), four other European ecotypes were selected on the basis that the climate of the region of origin was similar to the local projections (REMO A1B) for our site for the period 2071-2100. Seed material was obtained from these source regions, abbreviated below as Spain (ES), Italy (IT), Hungary (HU), and Bulgaria (BG), and from the northern part of the species' ranges (here: Sweden, SE) (Online appendix 1). For *Arrhenatherum elatius* and for *Festuca pratensis*, ecotypes were available from seed banks for all target regions. For *Holcus lanatus* and for *Alopecurus pratensis* only four ecotypes were available.

The target ecotypes were cultivated at the *Leibniz Institute of Plant Genetics and Crop Plant Research* (IPK) in Poel, Germany from February 2009 to April 2009. The seedlings were then transported to Bayreuth and individually planted into 4-liter plastic pots filled with sandy silt (pH 7.27, total C 1.89%, total N 0.15%, plant available NH_4^+ 1.79 mg l^{-1}, plant available NO_3^- 22.50 mg l^{-1}). From May to September 2009, the potted individuals were exposed to a warming treatment (warming and control) and to a drought treatment (extreme summer drought and control) in a split-plot design. The two climate treatments were fully crossed resulting in four climate manipulations (control, drought, warming, warming & drought) in three replications each. These experimental units were situated in 12 large rain-out shelters (7m x 10.5m). All shelters were covered with a transparent polyethylene sheet (0.2 mm, SPR5; Hermann Meyer KG, Rellingen, Germany) starting at a height of 80 cm and permitting nearly 90% penetration of photosynthetically active radiation. The duration of the drought treatment was based on signs of leaf senescence in more than 66% of the individuals and lasted 16 days for *H. lanatus*, 18 days for *A. pratensis* and *F. pratensis* and 19 days for *A. elatius*. The warming treatment was performed continuously until September 2009 using wind-shelters around and black floor-covers in the respective shelters, which increased the average air temperature at plant height by 1.5°C. Further details on the manipulations and the plant responses within the first year are provided in Beierkuhnlein *et al.* (2011).

The plants were overwintered outside in a sand-bed and exposed to ambient conditions for the remainder of the experiment. Three replicates per factorial group of ecotype and pretreatment were exposed to a late frost event in the night from 26th to 27th of May 2010 and another three replicates were used as a reference for the frost treatment. Uncharacteristically for our site, no

late frost event occurred naturally in 2010. Based on local climate data, a late frost event of -5°C was simulated for three hours by gradual cooling inside a cooler truck.

The temporal pattern of the chlorophyll content was monitored over five weeks after the late frost manipulation by weekly measurements using a SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc). Four leaves per plant and date were chosen randomly for the SPAD measurements and an average taken for the analyses. No SPAD-measurements were taken for the warming pretreatments. SPAD-readings were calibrated to foliar chlorophyll content for 20 leaves per species. SPAD readings were conducted for these samples and the leaves were then removed and kept at low temperatures without freezing for the immediate determination of the fresh weight. Afterwards, samples were frozen and kept at -29°C until chlorophyll extraction when the leaves were cooled in liquid nitrogen and ground in a ball mill while frozen. The chlorophyll was dissolved in 1 ml cold methanol for 30 min and shaken every 10 min. Extracts were centrifuged for 5 min at 4°C and 14000 rpm. The supernatant was stored in a freezer and warmed for 15 min at room temperature before the absorbance was measured at 645 and 663 nm using a spectrophotometer (DU-50, BECKMANN). The chlorophyll content per g fresh weight was calculated according to Porra (1989) as:

$$[chl]_{fwt}\left[\frac{mg}{g}\right] = \frac{(8{,}02 \cdot E_{663\,nm} + 20{,}2 \cdot E_{645\,nm})}{1000} \cdot \frac{V\,(MeOH\ + waterconte\ nt)[ml]}{freshweigh\ t[g]}$$

A linear regression between SPAD and chlorophyll content yielded significant correlations for all four species with r^2 values of 0.88 for *H. lanatus*, 0.70 for *A. pratensis*, 0.72 for *F. pratensis* and 0.68 for *A. elatius*.

Above-ground biomass was harvested twice in 2009 by clipping at a height of 3 cm above the soil in June and September, resembling local agricultural management routines for extensively used grasslands. In 2010, above-ground biomass was cut on 6th July, 42 days after the late frost manipulation. On all dates, the biomass was dried for 48 h at 70 °C, and weighed.

The minimum temperature for May for each ecotype retrieved from *Worldclim* (Hijmans et al. 2005) was used as an indicator of late frost occurrences. An ordinary-least-squares regression between this indicator and late frost tolerance, expressed as the percentage reduction in biomass in the late frost treatment as compared to the reference, was applied for each species in order to detect local adaptation to late frost events. Due to the low number of data points (six), significance level was set to 0.1 here. Significance was tested by a Monte-Carlo permutation procedure against a null model with zero slope in 1000 permutations (Jurasinski 2007).

Analyses of variance (ANOVA) combined with linear mixed effect models were applied for each species separately to test for the main and interactive effects of the four factors: ecotype, late frost, warming pretreatment and drought pretreatment. Concerning the chlorophyll content, repeated measures ANOVA were applied by including time as random factor in the mixed model (Pinheiro and Bates 2004). Data were log-transformed to improve the homogeneity of variances and the normality of residuals prior to analysis if necessary (Faraway 2006). In case of significant ecotype or interaction effects, post-hoc comparisons (Tukey's test) were run according to Hothorn et al. (2008). All statistical analyses were conducted with the software R 2.11.1 (R Development Core Team 2010) using the packages "nlme" and "multcomp".

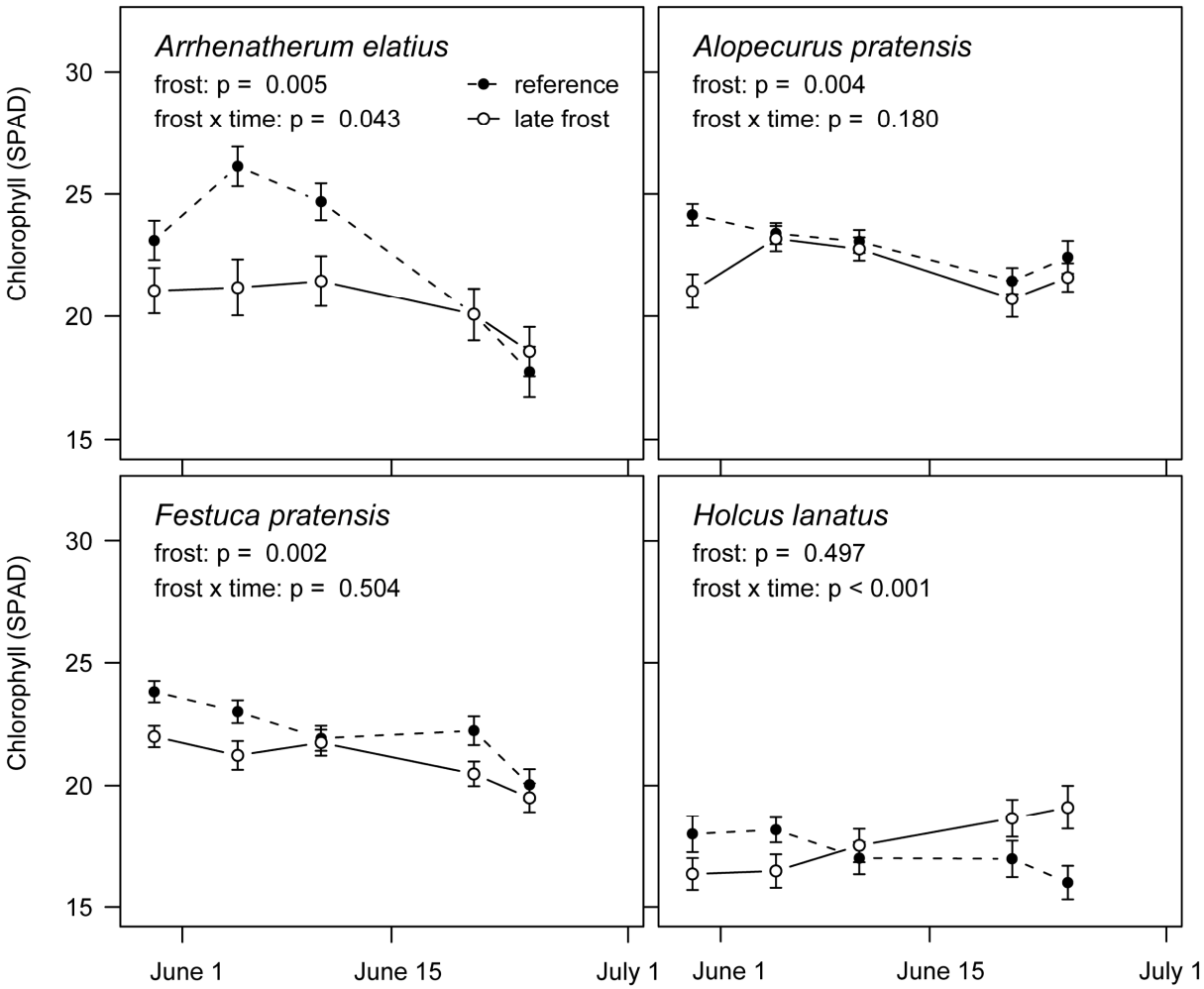

Figure 1: The late frost event significantly affected the chlorophyll content of the grasses. The late frost event was applied on May 26th, three days before the first chlorophyllmeter measurements. ANOVA-results of the mixed models are provided, for details see the text. Mean values and standard errors over 12 individuals and four or six ecotypes are displayed.

Results

Late Frost Events Affect Temperate European Grass Species

The late frost manipulation significantly reduced the chlorophyll content of three of the four grass species (Figure 1).

The only species which did not exhibit a significant late frost effect was *H. lanatus*. This species, however, showed a decreased chlorophyll content for two weeks, but this effect was inverted after four weeks (ANOVA: interaction between frost manipulation and time: $p < 0.001$). *A. elatius* and *A. pratensis* also showed a temporal development in the late frost effect, with the first species recovering after five weeks and the second species after only two weeks (Figure 1).

The temporal patterns of chlorophyll content were reflected in productivity. Above-ground biomass was reduced by 23% in *A. elatius* ($p < 0.001$), by 18% in *A. pratensis* ($p = 0.004$), and by 27% in *F. pratensis* ($p < 0.001$). No significant effect was found in *H. lanatus* (6%; $p = 0.409$) six weeks after the frost manipulation.

Local Adaptation

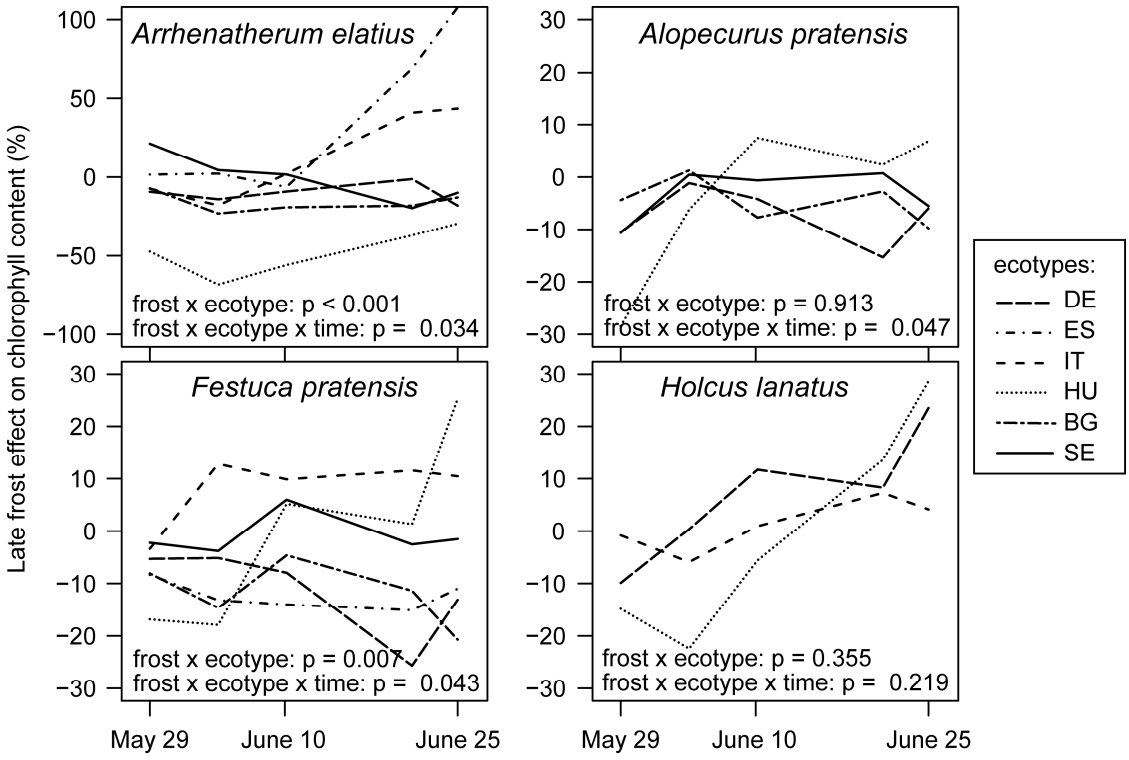

Figure 2: Ecotypes showed specific reactions in chlorophyll content to the late frost event over time. The late frost effect is given as the percental change in mean chlorophyll content between late frost manipulation and reference per ecotype. The late frost event was applied on May 26th, three days

before the first chlorophyllmeter measurements. ANOVA-results of mixed models for the interaction between the frost event and ecotype over time are provided.

Ecotypes differed in their late-frost sensitivity of chlorophyll content for *A. elatius* and *F. pratensis* (Figure 2). Temporal development of late-frost sensitivity was furthermore affected by the geographic origin of the ecotypes of these two species and also of *A. pratensis* (Figure 2). Only *H. lanatus* showed no significant differences between ecotypes concerning their late frost sensitivity, but we note that only three ecotypes could be analyzed for this species as the measurements of the fourth ecotype were lost.

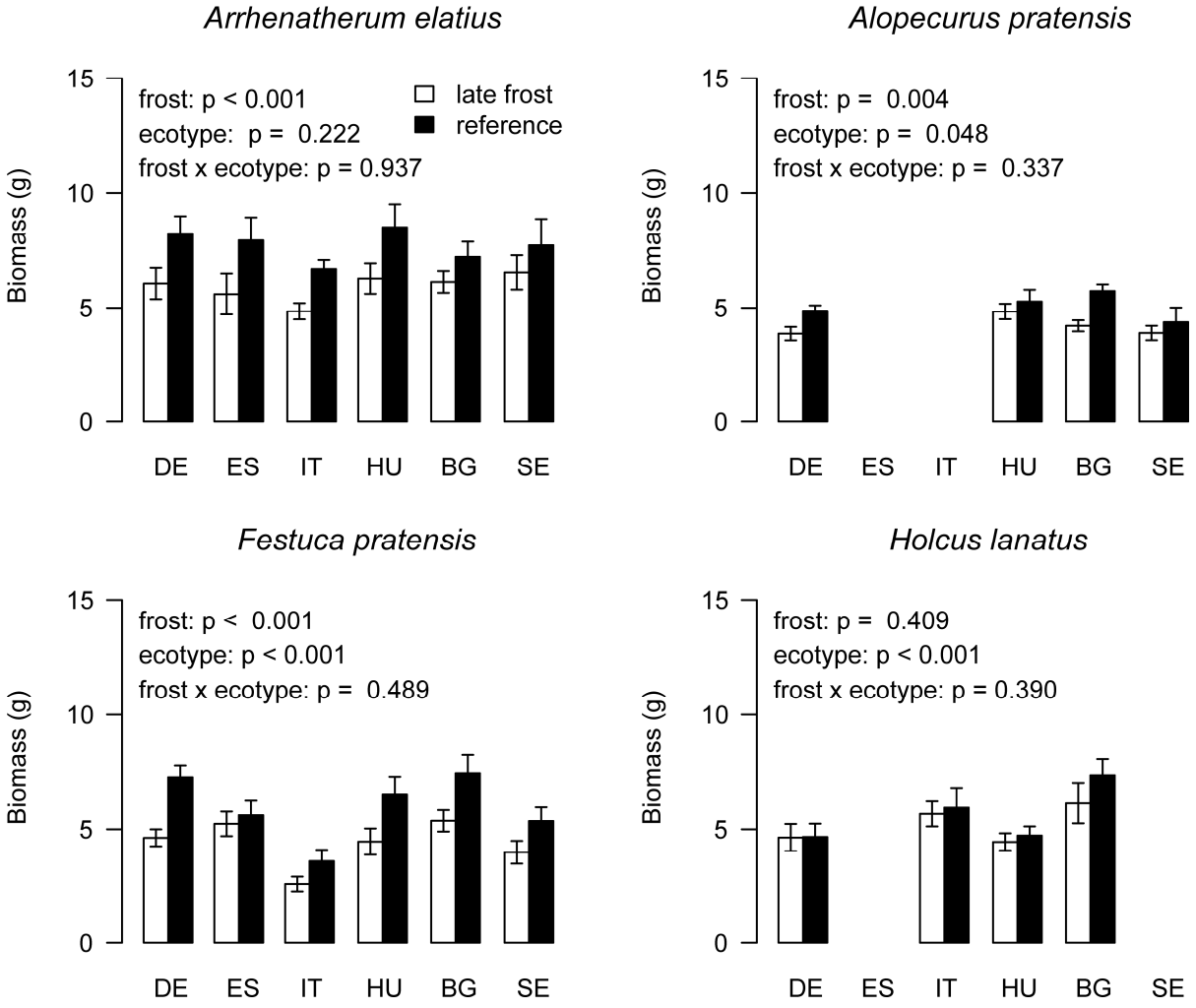

Figure 3: Aboveground biomass production per plant is affected by the late frost event. The late frost event was applied on May 26th, biomass harvests took place on July 6th. The ANOVA-results of linear models are provided. The mean values and standard errors over 12 individuals per bar are shown.

Furthermore ecotypes differed significantly in biomass production for all species except *A. elatius* (Figure 3). No significant differences in the effects of the late frost manipulation were detected between ecotypes for any of the species concerning biomass production (Table 1: interaction between late frost and ecotype). However, ecotypes from origins that are naturally

more prone to late frost events tended to be more tolerant to experimental late frost events, as indicated by the significant correlations between late-frost sensitivity and the minimum

Table 1: ANOVA results of the applied mixed models. Upper panel: biomass (fixed factors: ecotype, late frost, drought, warming; random factor: experimental unit). Lower panel: chlorophyll content (fixed factors: ecotype, late frost, drought, time; random factors: experimental unit, time). Note that chlorophyll content in *A. elatius* was not recorded in the drought pretreatment

		Arrhenaterum elatius		*Festuca pratensis*		*Holcus lanatus*		*Alopecurus pratensis*	
		F	p	F	p	F	p	F	p
biomass	late frost	**21.6**	**<0.001**	**31.4**	**<0.001**	0.7	0.409	**8.9**	**0.004**
	ecotype	1.4	0.221	**7.7**	**<0.001**	**7.6**	**<0.001**	**2.8**	**0.048**
	drought	**9.3**	**0.003**	**5.8**	**0.018**	**8.1**	**0.006**	3.0	0.090
	warming	**7.4**	**0.008**	**11.6**	**0.001**	**28.5**	**<0.001**	**5.4**	**0.024**
	late frost : ecotype	0.3	0.933	0.9	0.481	0.8	0.480	1.2	0.334
	late frost : drought	0.2	0.636	0.0	0.842	0.2	0.645	2.3	0.131
	late frost : warming	1.3	0.253	1.5	0.226	3.0	0.088	0.8	0.387
	late frost : ecotype : drought	**2.4**	**0.043**	0.3	0.910	2.6	0.058	1.9	0.136
	late frost : ecotype : warming	0.6	0.679	0.7	0.619	0.6	0.602	0.2	0.862
	late frost : drought : warming	0.1	0.850	2.1	0.149	**5.2**	**0.026**	0.4	0.531
chlorophyll	late frost	**8.2**	**0.005**	**9.5**	**0.002**	0.5	0.497	**8.3**	**0.004**
	ecotype	**10.0**	**<0.001**	**10.6**	**<0.001**	**16.6**	**<0.001**	**27.8**	**<0.001**
	drought			**7.6**	**0.006**	**7.4**	**0.007**	**6.0**	**0.015**
	time	6.2	0.089	**17.3**	**0.025**	1.0	0.386	2.9	0.183
	late frost : ecotype	**8.2**	**<0.001**	**3.3**	**0.007**	1.0	0.355	0.2	0.913
	late frost : drought			1.0	0.309	3.1	0.078	0.6	0.443
	late frost : time	**4.2**	**0.043**	0.4	0.504	**13.4**	**<0.001**	1.8	0.180
	late frost : ecotype : drought			**2.3**	**0.043**	1.2	0.293	0.8	0.479
	late frost : ecotype : time	**2.5**	**0.034**	**2.3**	**0.043**	1.5	0.219	**2.7**	**0.047**

temperature for May at the geographic origin of the ecotypes for *A. elatius* and *F. pratensis* (Figure 4). The non-significant relationships for the other two species might be related to the fact that fewer (four) data points (ecotypes) were available for these species.

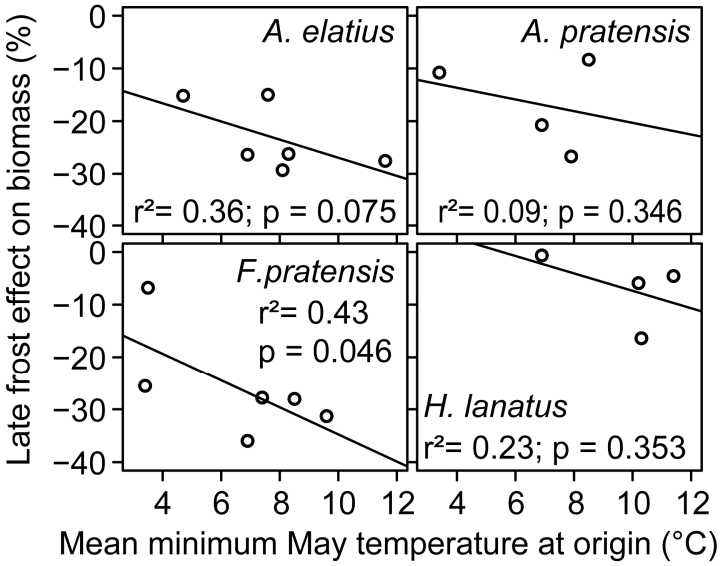

Figure 4: Late frost tolerance expressed as the percental change in biomass production between reference and frost manipulation as affected by the mean minimum temperature in May (obtained from worldclim; Hijmans *et al.* 2005).

Past Climate Experience Alter Late Frost Tolerance

Past climatic experience in terms of drought or warming affected late frost tolerance in three of the four species: For *A. elatius*, experience of extreme drought in the preceding growing season influenced biomass production ecotype-specifically (Table 1: interaction between late frost, ecotype and drought, Figure 5A). Here, previous exposure to drought generally tended to increase late frost tolerance, especially in the Bulgarian and German ecotypes, while in the reference treatment previous exposure to drought led to increased productivity in some ecotypes (DE, ES, IT, HU) and decreased productivity in others (BG, SE). *F. pratensis* exhibited a similar pattern of response (significant interaction between late frost, previous exposure to drought and ecotype) in terms of chlorophyll content. Note that chlorophyll content was not measured in the plants of the preceding warming manipulation. The third significant interaction between the late frost manipulation and previously experienced climatic conditions was found for *H. lanatus* (Table 1, Figure 5B). Here, the previous warming treatment reduced biomass production in combination with late frost, but not in combination with the reference treatment. Interestingly, the highest biomass production was found for those plants that had experienced drought in combination with increased

temperature in the previous year. This pattern, however, was irrespective of the late frost manipulation.

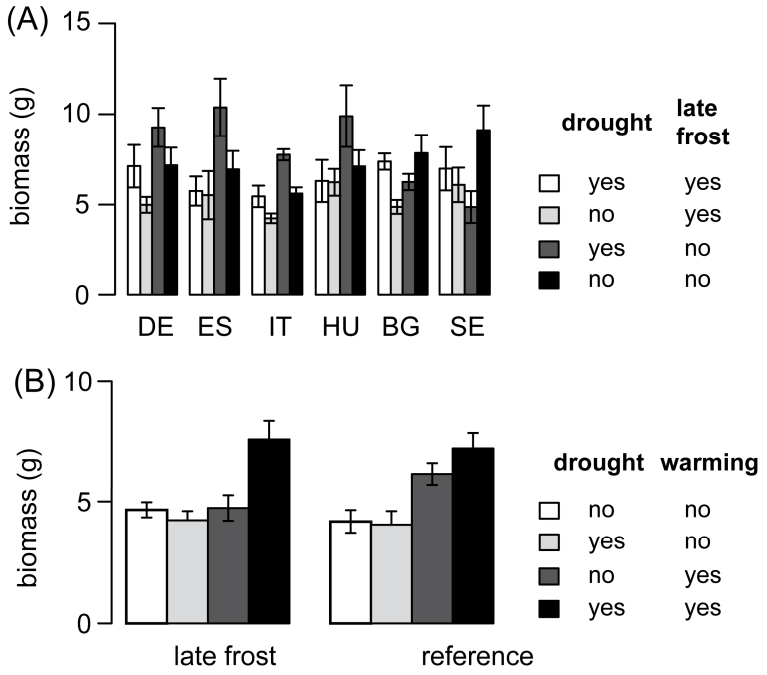

Figure 5: Climate conditions experienced in the preceding growing season can alter late frost tolerance. (A) Aboveground biomass production of *Arrhenatherum elatius* per provenance as affected by the late frost and previous drought (threefold interaction: $p = 0.043$). $n = 6$ per bar. (B) Aboveground biomass production of *Holcus lanatus* as affected by late frost, previous drought and previous warming (threefold interaction: $p = 0.026$). $n = 12$ per bar. Mean values and standard errors are displayed.

Discussion

Late Frost Events Affect Temperate European Grass Species

Important temperate European grass species decreased in biomass production on average by about 20% in response to a single late frost event lasting less than three hours. Late frost events are known to damage the seed production of temperate grass species (Hare 1995, Niemelainen 1991). Despite the fact that late frost events are irregular, but common in the temperate climate, the productivity of grass species in response to such events has not before been studied in detail. This is surprising as meadows and pastures cover large areas in temperate regions and are of high economic and ecological importance (Beierkuhnlein et al. 2011). Field observations (Gu et al. 2008, Inouye 2008) and model results (Rigby and Porporato 2008, Woldendorp et al. 2008, Bennie et al. 2010) indicate that the expected and already observed earlier onset of the growing season due to climate change may lead to an increased risk of late frost damage in spite of the general air warming trend.

The strong impact of late frost events on plant performance is due to the fact that they hit the vegetation at times when the plants have already lost their frost hardiness that protected them during the winter (Liu and Osborne 2008, Rapacz et al. 2004). Dehardening in response to

warming takes place within hours (Rapacz et al. 2000) and as a consequence the plants are left in a more vulnerable state. Frost events and other winter processes will continue to be important drivers of temperate vegetation performance even in a warmer climate (Kreyling 2010).

Local Adaptation

The selected ecotypes of our target species differed in their growth potential and also, at least for two species, in their sensitivity to late frost (Figures 2 and 3). Late-frost sensitivity furthermore correlated with the minimum temperatures for May at the geographic origins of the ecotypes (Figure 4), thereby indicating inherited local adaptation to late frost events. The selected wide-spread species can be expected to exhibit high genetic variation within and across their distribution area (Michalski et al. 2010), commonly displayed in strong local adaptation, especially to the climate (McKay et al. 2005, Joshi et al. 2001). For *F. pratensis*, leaf proteins are reported to accumulate differentially during cold acclimation between genotypes with distinct levels of frost tolerance (Kosmala et al. 2009). Home site advantages have also been found for *H. lanatus* with respect to the full set of climatic parameters in a transplanting experiment (Macel et al. 2007). Results from our experiment with respect to drought and warming, however, did not show any predictable patterns with superior performance by ecotypes with short climate transfers (Beierkuhnlein et al. 2011). This implies that late frost events have stronger evolutionary importance in these species than mean temperature or summer drought.

Climate has been a major driver of community compositions in the past and will continue to be in the future (Willis et al. 2010). Assisted colonization is therefore discussed as a means to protect rare and endangered species (Hoegh-Guldberg et al. 2008). Securing the functioning of ecosystems, however, might be another pressing need in times of fast climate change (Willis et al. 2010). The assisted colonization of pre-adapted ecotypes of key species with wide distributions might contribute to this goal without the need to introduce exotic species with unknown risks (Kreyling et al. 2011). Our results suggest that ecotypes of important and widely distributed grass species differ in their sensitivity to late frost. With respect to this single factor, the correlations between home site climate and late-frost sensitivity (Figure 4) suggest that the selection of ecotypes could possibly be based on the current climatic setting within the species ranges. However, uncertainties surrounding the future climate and the multitude of climatic parameters to select for imply that the search for best-adapted ecotypes may not be the best strategy. Rather, those management actions that promote genetic diversity

itself appear to be feasible, as genetic diversity enables organisms to continue to adapt and evolve to novel circumstances within few generations (Mace and Purvis 2008, Bischoff et al. 2010, Hoffmann and Sgrò 2011). However, potential negative impacts of this approach, such as the possibility that local populations and gene pools could be eradicated (McKay et al. 2005), should be taken into account prior to implementation.

Past Climate Experiences Alter Late Frost Tolerance

Climatic conditions experienced in the preceding growing season had the potential to alter plant performance in general and sensitivity to late frost in particular (Table 1). Although these effects only occurred in some species and some interactions of climatic parameters, they indicate the potential importance of preconditions. Walter *et al.* (2011) have shown that *A. elatius* exhibits a "drought memory" over an entire growing season with increased photoprotection during drought in those plants that had already experienced drought earlier in the growing season, before harvest and resprouting. Our results imply that such memory effects can also occur between growing seasons and between different climatic drivers, i.e., drought and late frost. We suggest that epigenetic changes and accumulation of signaling proteins or transcription factors (Aubin-Horth and Renn 2009, Bird 2007, Goh et al. 2003, Verhoeven et al. 2010, Bruce et al. 2007, Bossdorf et al. 2008) might be responsible for these findings. Both drought and frost tolerance rely on similar mechanisms (accumulation of low-molecular-weight osmolytes, which prevent cellular dehydration; Beck et al. 2007) which can explain the change in late frost tolerance after former exposure to drought.

Ultimately, species responses and within-species variability need to be regarded in the context of communities. The advantage of common garden experiments is their ability to detect the spectrum of species-specific responses. Nevertheless, there is a need to test the results obtained in communities where the competitive balance might be affected and thereby any differences between species amplified or buffered.

Conclusions

Productivity of ecologically and economically important grass species in Central Europe is significantly affected by late frost events. Earlier start of the growing season without change in the timing of late frost events might even lead to increased risk of damage in the future.

The target grass species exhibited local adaptation in late-frost sensitivity at the continental scale. The high within-species variability could be used in adaptation to climate change.

However, there is a clear need to investigate the response to other climatic drivers and management aimed at maximizing genetic diversity might yield better results than searching for the best-adapted genotype.

Acknowledgements

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References

Aubin-Horth, N. and Renn, S. C. P. 2009. Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. – Molec Ecol 18: 3763–3780.

Beck, E. H. et al. 2007. Specific and unspecific responses of plants to cold and drought stress. – J Biosci 32: 501–510.

Beierkuhnlein, C. et al. 2011. Ecotypes of European grass species respond differently to warming and extreme drought. – J Ecol: doi: 10.1111/j.1365-2745.2011.01809.x.

Bennie, J. et al. 2010. Predicting spatial and temporal patterns of bud-burst and spring frost risk in north-west Europe: the implications of local adaptation to climate. – Glob Change Biol 16: 1503–1514.

Bird, A. 2007. Perceptions of epigenetics. – Nature 447: 396–398.

Bischoff, A. et al. 2010. The importance of plant provenance and genotypic diversity of seed material used for ecological restoration. – Restor Ecol 18: 338–348.

Bossdorf, O. et al. 2008. Epigenetics for ecologists. – Ecol Lett 11: 106–115.

Bruce, T. J. et al. 2007. Stressful "memories" of plants: Evidence and possible mechanisms. – Plant Sci 173: 603–608.

Cromey, M. G. et al. 1998. Effects of frost during grain filling on wheat yield and grain structure. – New Zeal J Crop Hort Sci 26: 279–290.

Eccel, E. et al. 2009. Risk of spring frost to apple production under future climate scenarios: the role of phenological acclimation. – Int J Biometeorol 53: 273–286.

Faraway, J. J. 2006. Extending the Linear Model with R - Generalized Linear, Mixed Effects and Nonparametric Regression Models - Chapman & Hall/CRC.

Goh, C. H. et al. 2003. Stress memory in plants: a negative regulation of stomatal response and transient induction of rd22 gene to light in abscisic acid-entrained *Arabidopsis* plants. – Plant J 36: 240–255.

Gu, L. et al. 2008. The 2007 Eastern US spring freeze: increased cold damage in a warming world? – Bioscience 58: 253–262.

Hare, M. D. 1995. Frost severity, frost duration, and frost protectants affect tall fescue seed production. – New Zeal J Agr Res 38: 79–83.

Hijmans, R. J. et al. 2005. Very high resolution interpolated climate surfaces for global land areas. – Int J Climatol 25: 1965–1978.

Hoegh-Guldberg, O. et al. 2008. Assisted colonization and rapid climate change. – Science 321: 345–346.

Hoffmann, A. A. and Sgrò, C. M. 2011. Climate change and evolutionary adaptation. – Nature 470: 479–485.

Hothorn, T. et al. 2008. Simultaneous inference in general parametric models. – Biometrical J 50: 346–363.

Hufford, K. M. and Mazer, S. J. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. – Trends Ecol Evol 18: 147–155.

Humphreys, M. W. et al. 2006. A changing climate for grassland research. – New Phytol 169: 9–26.

Inouye, D. W. 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. – Ecology 89: 353–362.

Joshi, J. et al. 2001. Local adaptation enhances performance of common plant species. – Ecol Lett 4: 536–544.

Jurasinski G (2007) simba: A Collection of functions for similarity calculation of binary data. R package version 0.2-5. http://www.r-project.org.

Kosmala, A. et al. 2009. Identification of leaf proteins differentially accumulated during cold acclimation between *Festuca pratensis* plants with distinct levels of frost tolerance. – J Exp Bot 60: 3595–3609.

Kreyling, J. 2010. Winter climate change: a critical factor for temperate vegetation performance. – Ecology 91: 1939–1948.

Kreyling, J. et al. 2011. Assisted colonization - a question of focal units and recipient localities. – Restor Ecol 19: 433-440.

Liu, M.-Z. and Osborne, C. P. 2008. Leaf cold acclimation and freezing injury in C-3 and C-4 grasses of the Mongolian Plateau. – J Exp Bot 59: 4161–4170.

Mace, G. M. and Purvis, A. 2008. Evolutionary biology and practical conservation: bridging a widening gap. – Molec Ecol 17: 9–19.

Macel, M. et al. 2007. Climate vs. soil factors in local adaptation of two common plant species. – Ecology 88: 424–433.

McKay, J. K. et al. 2005. "How local is local?" - A review of practical and conceptual issues in the genetics of restoration. – Restor Ecol 13: 432–440.

Michalski, S. G. et al. 2010. Evidence for genetic differentiation and divergent selection in an autotetraploid forage grass (*Arrhenatherum elatius*). – Theor Appl Genet 120: 1151–1162.

Niemelainen, O. 1991. Factors affecting panicle production of cocksfoot (*Dactylis glomerata* L) in Finland .4. response to frost at panicle growth-phase. – Ann Agr Fenn 30: 63–172.

Oberdorfer, E. 2001 Pflanzensoziologische Exkursionsflora. Eugen Ulmer Verlag, Stuttgart.

Ofir, M. and Kigel, J. 2010. Ecotypic variation of summer dormancy relaxation associated with rainfall gradient in the geophytic grass *Poa bulbosa*. – Ann Bot 105: 617–625.
Pinheiro, J. C. and Bates, D. M. 2004. Mixed-effects models in S and S-PLUS - Springer.
Porra, R. J. et al. 1989. Determination of accurate extinction coefficients and simultaneous-equations for assaying chlorophyll-a and chlorophyll-b extracted with 4 different solvents - verification of the concentration of chlorophyll standards by atomic-absorption spectroscopy. – Biochimt Biophys Acta 975: 384–394.
R Development Core Team 2010. [R: A Language and Environment for Statistical Computing. R version 2.11.1 - R Foundation for Statistical Computing. ISBN 3-900051-07-0, URL http://www.R-project.org.
Rapacz, M. et al. 2004. Changes in cold tolerance and the mechanisms of acclimation of photosystem II to cold hardening generated by anther culture of *Festuca pratensis* x *Lolium multiflorum* cultivars. – New Phytol 162: 105–114.
Rapacz, M. et al. 2000. Frost de-acclimation of barley (*Hordeum vulgare* L.) and meadow fescue (*Festuca pratensis* Huds.). Relationship between soluble carbohydrate content and resistance to frost and the fungal pathogen *Bipolaris sorokiniana* (Sacc.) Shoem. – Ann Bot 86: 539–545.
Rigby, J. R. and Porporato, A. 2008. Spring frost risk in a changing climate. – Geophys Res Lett 35: L12703.
Sandve, S. R. et al. 2011. Molecular mechanisms underlying frost tolerance in perennial grasses adapted to cold climates. – Plant Sci 180: 69–77.
Verhoeven, K. J. F. et al. 2010. Stress-induced DNA methylation changes and their heritability in asexual dandelions. – New Phytol 185: 1108–1118.
Walter, J. et al. 2011. Do plants remember drought? Hints towards a drought-memory in grasses. – Environ Exp Bot 71: 34–40.
Willis, K. J. et al. 2010. Biodiversity baselines, thresholds and resilience: testing predictions and assumptions using palaeoecological data. – Trends Ecol Evol 25: 583–591.
Woldendorp, G. et al. 2008. Frost in a future climate: modelling interactive effects of warmer temperatures and rising atmospheric [CO2] on the incidence and severity of frost damage in a temperate evergreen (*Eucalyptus pauciflora*). – Glob Change Biol 14: 294–308.

Online Appendix 1: Origin of the ecotypes of *A. elatius*, *F. pratensis*, *H. lanatus* and *A. pratensis* used in the experiment. Mean Minimum Temperature in May (Tmin May), Mean Annual Temperature (MAT) and Mean Annual Precipitation (MAP) represent long-term conditions (Hijmans et a. 2005). Accession responds to the code of the seed bank at IPK Poel (codes starting with GR are from the IPK seed bank, codes with PI from USDA-ARS-GRIN, codes with NordGen, Bf and RCAT are from Eurisco.

		Location	Accession	Latitude	Longitude	Elevation (m a.s.l.)	Tmin May (°C)	MAT (C°)	MAP (mm)
A. elatius	DE	Blaufelden, Hohenloher Ebene	GR 12752	49°17'46"N	09°58'25"E	460	6.9	8.3	732
	SE	Uppsala	PI 235543	59°51'25"N	17°38'22"E	20	4.7	5.7	551
	ES	Montalban	PI 234465	40°50'07"N	00°47'55"E	900	8.1	11.0	450
	IT	Rizzolo, Emilia-Romagna	GR 12733	44°55'12"N	09°44'46"E	110	11.6	12.9	739
	HU	Lókút	RCAT064783	47°12'00"N	17°52'00"E	440	8.3	8.9	621
	BG	NA	BGR: 2008-ARR-EL-1	42°00'00"N	24°50'00"E	NA	7.6	NA	NA
F. pratensis	DE	Blaufelden, Hohenloher Ebene	GR 12753	49°17'46"N	09°58'25"E	460	6.9	8.3	732
	SE	Mårtenstorpet, Grängesberg	NordGen: 1191	60°00'00"N	15°00'00"E	350	3.4	4.3	738
	ES	San Anton	Bf 1592	42°37'08"N	00°09'47"W	1250	3.5	6.8	989
	IT	Lago Nero, Ferriere Piacenza	Bf 1078	44°33'00"N	09°27'00"E	1600	7.4	8.5	981
	HU	Vérteskozma (Gánt)	RCAT040707	47°27'00"N	18°28'00"E	270	9.6	10.2	571
	BG	Dolna Banya	GR 6976/99	42°19'05"N	23°45'07"E	710	8.5	9.6	585
H. lanatus	DE	Blaufelden, Hohenloher Ebene	GR 12750	49°17'46"N	09°58'25"E	460	6.9	8.3	732
	IT	Torrano, Emilia-Romagna	GRA 312	44°53'33"N	09°41'20"E	160	11.4	12.6	758
	HU	Horváthertelend	RCAT040972	46°10'00"N	17°55'00"E	200	10.2	10.9	675
	BG	Mihiltsi	GR 6632/00	42°31'19"N	24°48'52"E	330	10.3	10.9	581
A. pratensis	DE	Blaufelden, Hohenloher Ebene	GR 12751	49°17'46"N	09°58'25"E	460	6.9	8.3	732
	SE	Mårtenstorpet, Grängesberg	NordGen: 1183	60°00'00"N	15°00'00"E	350	3.4	4.3	738
	HU	Lókút	RCAT064581	47°12'00"N	17°52'00"E	440	8.5	8.9	621
	BG	Iskar Dam	GR 6635/00	42°26'29"N	23°35'20"E	810	7.9	9.0	593

6.8. Manuscript 8:

Intraspecific diversity abets chances of high yields under different climatic conditions – a modelled example from provenance trial data

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Keywords

Fagus sylvatica, Pinus nigra, insurance hypothesis, portfolio effect, within-species diversity, genetic diversity, phenotypic plasticity, common garden

Abstract

The enhancement of genetic diversity and phenotypic plasticity of forests stands, e.g. by mixing stress-adapted provenances (e.g. from more drought-prone regions within the distribution range) with local provenances, has been recently proposed as adaptation mechanism to adverse impacts of climate change. This might be seen as a *portfolio* or *insurance effect*, against the negative impacts of diverse climatic stressors. However, there is no clear evidence whether an artificial enhancement of intraspecific diversity by "provenance-mixing" can maintain high yields under favourable conditions, while securing ecosystem functioning und persistence under extreme conditions.

We employed a sampling procedure (maximum selection) to investigate potential benefits and the impacts of a mixing of provenances in one stand on performance under different climate conditions, with data from a full factorial common garden experiment with two European tree species. Results suggest that there is a positive effect of provenance diversity on expected yield.

Introduction

The velocity of climate change, especially a more frequent occurrence of climatic extremes like drought periods, may exceed the ability of long-living organism like trees to adapt in a timely manner in terms of natural dispersal (Thomas *et al.* 2004; Chen *et al.* 2011; Zhu *et al.* 2012) or evolutionary adaptation processes (Lindner *et al.* 2010). This will lead to a range reduction for many species and at the same time a loss of genetic diversity (Alsos *et al.* 2012). Declining genetic diversity on the other hand decreases a species potential to adapt to changing environmental conditions (Jump *et al.* 2009). Therefore counteracting silvicultural practices that aim on supporting dispersal and facilitating adaptability may become necessary in order to maintain ecosystem functioning and thus the provision of forest ecosystem goods and services (Kolström *et al.* 2011).

Forest management has to deal with the risk of uncertain future growing conditions, and a good forest management strategy should be designed to minimize risk and maximize the expected net present value of forest stands (Yousefpour & Hanewinkel 2009). However, unlike economics, forest management has hardly introduced risk management processes into decision making (Knoke *et al.* 2005; Hanewinkel *et al.* 2011). In economics the *portfolio effect*, described by Markowitz (1952), states that a diversification of investments reduces the risk of a complete loss of profits. In ecology, a similar concept, the *insurance hypothesis*, describes the positive effect of biodiversity on ecosystem functioning and reliability, as the higher the number of species, the more likely the function of a failing species can be adopted by other species in the system (Yachi & Loreau 1999). In forestry the idea of conversion of forests from single-species stands into mixed forests as a means of risk reduction became popular again over the last decades (Knoke *et al.* 2005). However, intraspecific diversity just recently came into focus of forest science and management.

Up to now the introduction of exotic species from warmer or drier climates or of ecotypes of native species from warmer or drier regions within the distribution range has mainly been proposed to counter the negative impacts of rapid climate change (Bolte *et al.* 2009; Kreyling *et al.* 2011; Pedlar *et al.* 2011). Especially highly-adapted populations from the dry edges of the distribution ranges come into focus in search of drought-resistant ecotypes (Rose *et al.* 2009; Kreyling *et al.* 2011; Wortemann *et al.* 2011). Then again, recent studies revealed a potential trade-off between drought adaptation and phenotypic plasticity, what means that concentrating on single drought adapted species or ecotypes might lead to yield reductions

under favourable conditions and therefore limit the effectiveness of such measures (Richter *et al.* 2012; Thiel *et al*. subm.).

Phenotypic plasticity is genetically controlled (Nicotra *et al.* 2010), so high levels of genetic diversity are likely to include genotypes that allow plastic responses. On the other hand it seems that high phenotypic plasticity also fosters rapid genetic adaptation to changing environments (Lande 2009; Nicotra *et al.* 2010). Under very extreme conditions however, non-plastic highly adapted ecotypes may secure the persistence of a population. Furthermore, resistance to one abiotic factor does not necessarily imply a resistance to other environmental factors (Kreyling *et al.* 2012b; Thiel *et al.* 2012).

A mixing of different provenances, i.e. ecotypes from different regions within the distribution range of a species that might have undergone local adaptations to the climate conditions at the origin, has therefore been suggested recently by several authors (Kolström *et al.* 2011; Thiel *et al.* 2012; Frascaria-Lacoste & Fernández-Manjarrés 2012) as a promising silvicultural measure, to dampen the adverse effects of climate change on forest ecosystem functioning and the provision of goods and services and to enhance the adaptive potential of a species (Sgrò *et al.* 2011). However, there is no clear evidence whether an artificial enhancement of genotypic diversity and phenotypic plasticity, e.g. by intermixing highly –adapted and plastic provenances from different climatic regions, may maintain high yields under favourable conditions, while securing ecosystem functioning und persistence under extreme conditions.

Here, we focussed on two species: *Pinus nigra*, a generally drought-resistant species (Isajev *et al.* 2004) with a patchy (sub-) Mediterranean distribution range, which is a potential candidate to replace climate change-threatened conifer species like *Picea abies* in Central Europe. And *Fagus sylvatica*, which is the dominant native forest tree in Central Europe yet considered highly threatened in the face of climate change (Gessler *et al.* 2007; Ohlemuller *et al.* 2006). Therefore the translocation of ecotypes from within its distribution range is discussed (Bolte & Degen 2010; Kreyling *et al.* 2012a). We employed a sampling procedure (maximum selection) to investigate potential benefits and the impacts of high intraspecific variability, i.e. a *portfolio* of provenances in one stand, on growth and risk of yield losses under drought and favourable conditions. Within this sampling procedure, trees with the highest increment survived (maximum selection). Natural regeneration in forests, however, can follow, under certain circumstances, a more random path of recruitment (lottery sampling) (Lamont & Witkowski 1995; Busing & Brokaw 2002), which was also tested. Data was taken from a full factorial common garden experiment, in which seedlings of eight provenances of *P. nigra* and

seven of *F. sylvatica,* were exposed to drought conditions (Kreyling *et al.* 2012a; Thiel *et al.* 2012). Furthermore artificial data sets were created to search for relations between basic properties of data sets and modelling results.

Based on the positive impacts of genetic diversity and phenotypic plasticity on adaptive capacity (Lande 2009; Nicotra *et al.* 2010), we hypothesized that (1) the more provenances were included the smaller the yield losses under drought and control conditions, but (2) that the strength of this effect depends on basic properties (e.g. differences in within- and among-provenance variance and medians) of the underlying data sets. Further we aimed to demonstrate that the decision for an assumption about the selection process of individuals during their establishment might influence the results and subsequently implications for management decisions.

Methods and Material

Experimental data

The data used in this study are derived from a common garden experiment (EVENT 3) in Bayreuth, Germany, in the Ecological-Botanical gardens of the University of Bayreuth. Seedlings of eight provenances of *P. nigra* and seven of *F. sylvatica* were provided by the Bavarian Institute for Forest Seeding and Planting in Teisendorf, Germany. The seedlings were planted in pots and exposed to drought and control conditions in a full factorial experimental design 2009/2010 (*P. nigra*) and 2010 (*F. sylvatica*). The *P. nigra* data-set includes 42 replicates per provenance and treatment, whereas the *F. sylvatica* data-set comprises 18 replicates per treatment and provenance. The climate manipulations consisted of two treatments. In the control treatment individuals of both species were irrigated according to the local daily 30-year average precipitation. The application was done twice a week with collected rain water. The drought treatment was comprised of a 42-day period, starting May 27th 2009, without irrigation, which represents a local drought event with a statistical reoccurrence probability of 1000 years for *P. nigra*. For *F. sylvatica* a 64-day period, defined by the response of the plants, constituted the drought treatment. (Kreyling *et al.* 2012a) and (Thiel *et al.* 2012) provide detailed information on provenances, experimental set-up and climate manipulations.

The height of the plants was recorded before and at the end of the growing season. The relative increment rate was determined as the difference between those two in percent.

An Analysis of Variance (ANOVA) was applied to test for the main and interactive effects of the factors drought and provenance on relative height growth, in order to describe the structure and properties of the experimental data (e.g. differences in the performance of provenances). The data was tested for homogeneity of variance and normality of residuals prior to analysis and were log-transformed or square-root-transformed, if necessary. A post-hoc test (Tukey HSD) was conducted, in case of significant provenance effects, in order to depict pairwise differences between provenances.

Artificial data

In addition to the experimental data, an artificial data set was created in order to facilitate the interpretation of simulation results. Those data sets could also be interpreted as provenances of one species growing under several conditions causing different responses of the species in terms of growth increment. The artificial data sets consisted of seven provenances characterized by mean growth and variation (normal distributed data). Means of the provenances were random numbers drawn from a normal distribution. Variation within each provenance was the same for all of them (Fig. 3a). This basic data set (Fig. 3a) was modified to assess the influence of data structure on the results. First, means of the provenances were multiplied with a constant to increase differences between them (Fig. 3b, constant was 10). Further the basic data set was modified by creating an “over-performing” provenance with significantly higher increment rates than others (Fig. c, adding 10 to provenance 7). The next data scenario was the same as in Fig. 3c, i.e. one “over-performer”, but also contained an “under-performing” provenance (Fig. 3d). The same was done with two “over-performers” (Fig. 3e), where one data scenario again contained an “under-performer” (Fig. 3f). On those data sets the maximum-sampling described below was applied in the same way as for the experimental data.

Maximum-selection

The maximum selection mimicked an experiment where several individuals are hypothetically planted. The best performing individual (best growing) of each plantation was supposed to survive and its growth was measured. The underlying assumption was that growth is closely linked to competitiveness and survival.

In the sampling procedure the first step was to create a data subset for each possible combination of provenances. For *P. nigra* with eight provenances this resulted in 255 subsets and for *F. sylvatica* with seven provenances in 127 subsets.

Then, maximum selection was applied for five randomly selected individuals in each subset. This was repeated 5000 times for each subset. From those values the medians of the minimum, median, maximum and the quantiles 0.05, 0.25, 0.75, and 0.95 were calculated (from here on, when spoken of "quantiles", always the median of these quantiles from the sampling procedure is meant).

The results of the sampling procedure were grouped according to the number of provenances in the subsets (Fig. 2). Thereby for pine eight and for beech seven groups were created. Number of data points within the groups differs according to the number of possible combinations, which has to be considered in interpretations.

Maximum-selection vs. Lottery- Sampling

In order to show the effect of the chosen selection process we compared the often applied lottery-selection with maximum selection at the example of the *P. nigra* data set. The same procedure as for the maximum selection process was applied and the results were grouped according to the number of provenances in the subset, again. For the lottery-selection instead of the best performing individual out of five randomly chosen individuals just one random individual was selected. For each provenance maximum-selection and lottery-selection was applied and to show the effect of the chosen method on evaluating the relative performance of the provenances they were ranked.

Results

Experimental data

Provenances of *P. nigra* showed significant differences in height growth under the drought treatment (ANOVA: $p = 0.046$, $F = 2.1$; Fig. 1a), yet not under the control-treatment (ANOVA: $p = 0.093$, $F = 1.8$) or across treatments (ANOVA: $p = 0.119$, $F = 1.6$). Drought significantly reduced height growth (ANOVA: $p < 0.001$, $F = 85.3$) and the provenances differed in their response to drought (ANOVA: interaction between provenance and drought: $p = 0.018$, $F = 2.4$). Mainly two provenances were responsible for the significant differences

under the drought treatment; the Hungarian provenance (HU) grew exceptionally well, whereas the German provenance had the lowest increment rates (Fig. 1a).

F. sylvatica exhibited significant differences between provenances, both under drought (ANOVA: $p = 0.014$, $F = 2.8$; Fig. 1b) and control (ANOVA: $p < 0.001$, $F = 4.7$) conditions, as well as across treatments (ANOVA: $p < 0.001$, $F = 5.9$). Drought negatively impacted height growth for *F. sylvatica* (ANOVA: $p = 0.001$, $F = 11.1$), however, differences in drought sensitivity between the provenances could not be detected (ANOVA: $p = 0.394$, $F = 1.1$). Within the control treatment two Bulgarian (BG1, BG2) and one German provenance (DE1) showed high increment rates, whereas the DE2 (Germany) provenance grew significantly worse than these three. Under drought conditions, again DE1, with the highest growth rate, significantly differed from the Hungarian provenance with the lowest growth rate (Fig. 1b).

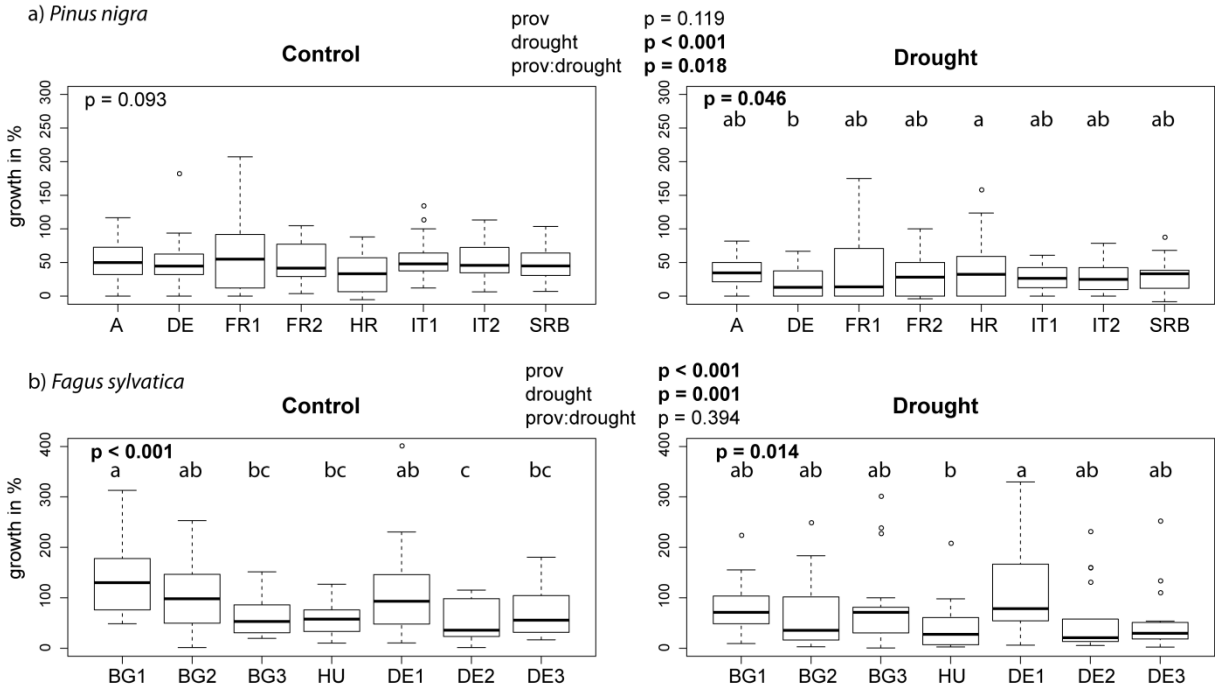

Figure 1: Experimental data with ANOVA results. a) Growth rates of *Pinus nigra* provenances in % for control and drought treatment b) Growth rates of *Fagus sylvatica* provenances for control and drought treatment. Lower case letters represent significant differences between provenances as revealed by a post-hoc test.

Maximum-sampling of the experimental data showed that for *P. nigra*, for both control and drought treatment, only the upper quantiles (0.95, maximum) increase with an increasing number of provenances (Fig. 2a). However, the increase in expected growth maxima (maximum, 0.95) culminated when 5-6 provenances were included, more provenances did not

have additional positive effects. All other quantiles (minimum, 0.05, 0.25, 0.5, 0.75) stayed remarkably stable, independent of the number of provenances included. For the drought treatment, a slight increase in median and its upper quartile (0.5, 0.75) from the subsets with one to subsets with two provenances became visible (Fig. 2a).

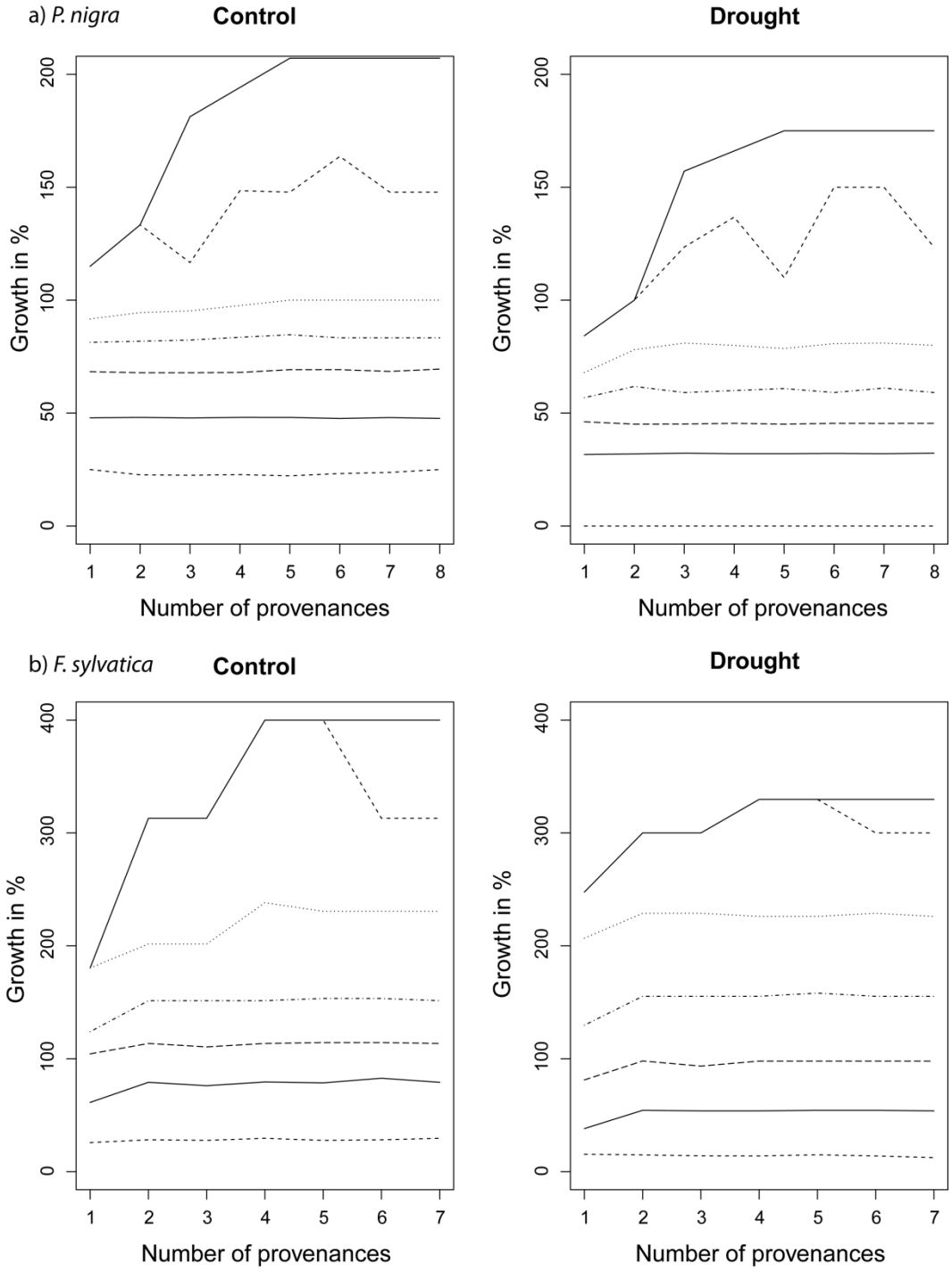

Figure 2: Results of the maximum selection procedure for a) *P. nigra* and b) *F. sylvatica*. Lines represent the medians of the quantiles (0, 0.05, 0.25, 0.5, 0.75, 0.95, 1) of 5000 repetitions of maximum selection grouped according to number of provenances included.

For *F. sylvatica* the maximum sampling procedure yielded similar results. Here, the slight increase from subsets with one to subsets with two provenances could be demonstrated for the quartiles 0.05 to 0.75 for both treatments (Fig. 2b). Again the expected maximum growth (0.95, and maximum) increased with increasing number of provenances. The control treatment, with three "over-performing" provenances (Fig. 1b) showed a steeper increase in expected maximum growth than the drought treatment with only one "over-performer". Subsets with more than four provenances did not yield additional positive effects on expected growth maxima. On the contrary, subsets with six or more provenances showed a decrease of the median of the 0.95 quartile (Fig. 2b).

Artificial data

The artificial data set with low variance between the provenances resulted in a stable behaviour of all quantiles, except a slight increase of the expected maximum growth (Fig. 3a). A higher variance between the provenances led to a stronger increase of the upper quartiles (0.5, 075, 0.95, maximum; Fig 3b), with a culmination at subsets with four or more provenances included. Adding one "over-performer" to the low variance data set, caused a steep increase of the upper quantiles (0.5, 0.75, and 0.95, maximum) of expected growth response from subsets with three to subsets with four provenances (Fig. 3c), with a slight decrease of the median with five or more provenances included in the subset. Adding one "under-performer" provenance did not change these results mentionable (Fig. 3d). The data set with two "over-performers" exhibited a steep increase of expected growth in almost all quantiles (0.05 – maximum) from single-provenance subsets to subsets with two provenances and a stable performance from that point on (Fig. 3e). Again, adding one "under-performer" did not change the results (Fig. 3f).

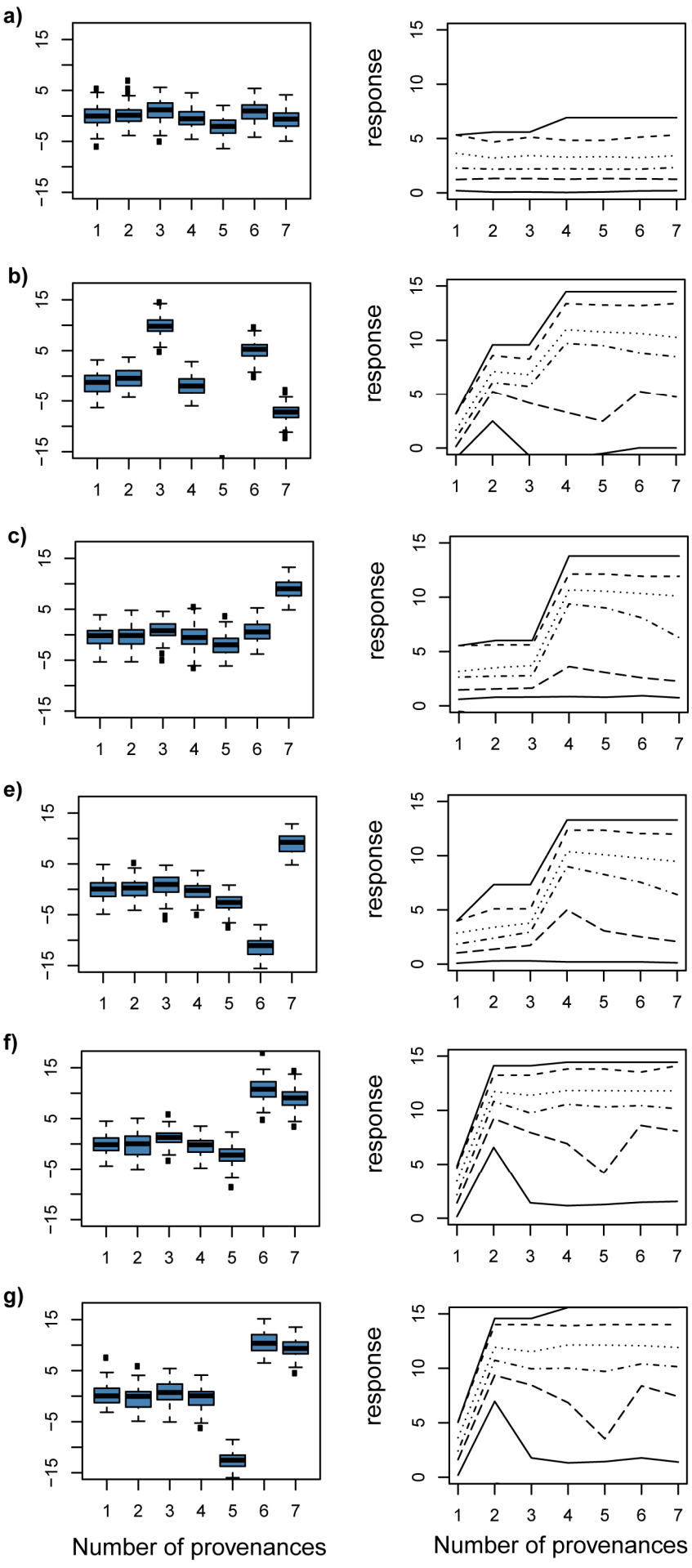

Figure 3: Artificial data sets. a) low variance, b) increased inter-provenance variance, c) low variance data set with one "over-performer", d) with one "over-performer" and one "under-performer", e) with two "over-performers" and f) with two "over-performers" and one "under performer" on the right side, and on the left side the corresponding results of maximum selection. Lines represent the medians of the quantiles (0, 0.05, 0.25, 0.5, 0.75, 0.95, 1) of 5000 repetitions of maximum selection grouped according to number of provenances included.

Maximum vs. Lottery- Sampling

Lottery selection in comparison to maximum selection yielded the same results for the expected maximum growth, yet all other quantiles are lower. (Fig. 4). Furthermore the ranks in expected median growth of the provenances changed between the methods, especially under the drought treatment, where provenances with a high variance (Fig. 1a) seem to profit from the maximum-sampling method. Furthermore the ranks change between the treatments (Tab 1).

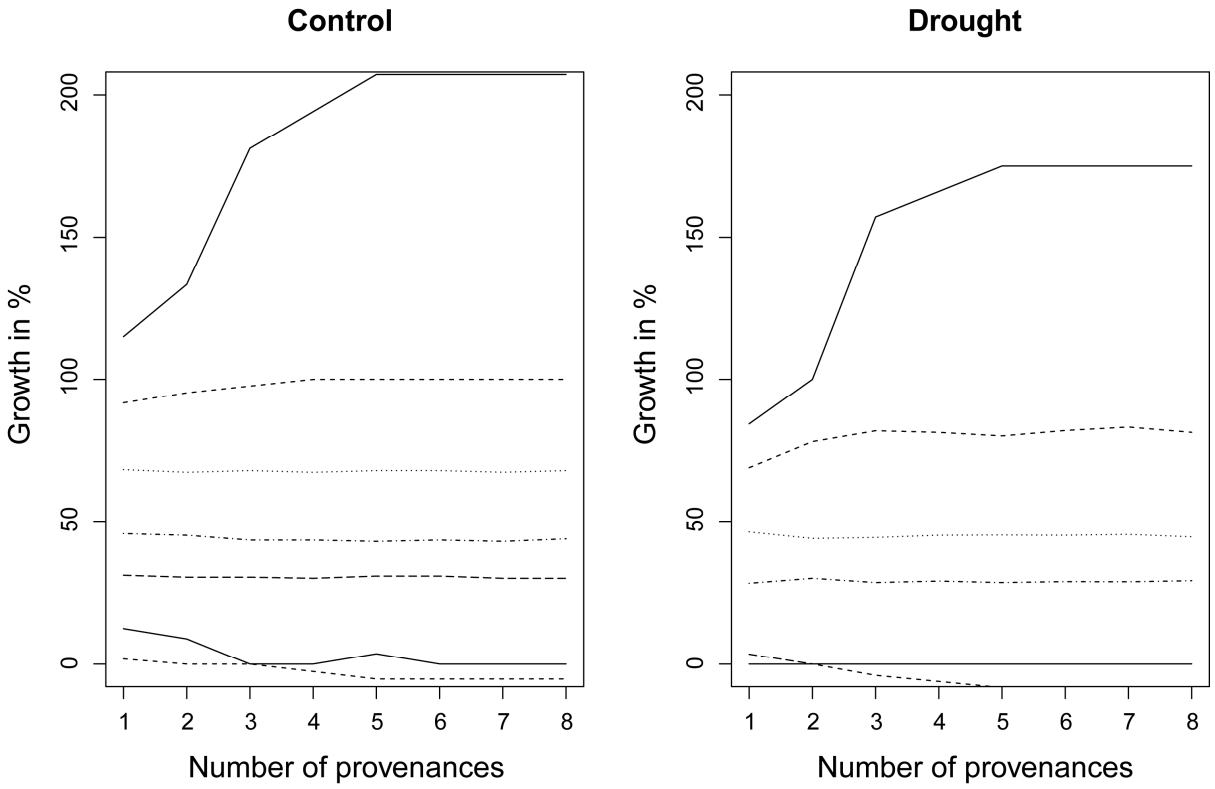

Figure 4: Results of the lottery (random) selection procedure for *P. nigra*. Lines represent the medians of the quantiles (0, 0.05, 0.25, 0.5, 0.75, 0.95, 1) of 5000 repetitions of maximum selection grouped according to number of provenances included

	A	DE	FR1	FR2	HR	IT1	IT2	SRB
				control				
Lottery selection	2	4	1	7	8	3	5	6
Maximum selection	5	8	1	3	7	6	2	3
				drought				
Lottery selection	1	8	7	5	3	4	6	2
Maximum selection	4	5	1	3	2	7	7	6

Table 5: Ranks of expected median growth fort he different provenances, treatments and selection method. 1 represents highest median growth, 8 the smallest.

Discussion

Both, the results from the experimental data, and the results of the artificial data sets, suggest that "provenance-mixing" can have positive impacts on expected yields under various conditions, as proposed e.g. by Kolström et al. (2011), Thiel et al. (2012) or Frascaria-Lacoste and Fernández-Manjarrés (2012). Particularly the chance of high yields can be increased by provenance mixing whereas the risk of low yields is not attenuated. Promoting the diversity of provenances might be one strategy to cope with adverse impacts of climate change, considering the associated uncertainties (Brang et al. 2008).

However, positive impacts of an increasing number of provenances became visible in the model, when a high inter-provenance variance existed. This supports the hypothesis that the strength of this diversity effect depends on properties of the input data. In the case of the artificial data the low variance option did not yield any substantial improvements in yield expectations, what is obvious, when adding more provenances does not result in additional variability. As soon as the differences between the provenances became more distinct (e.g. caused by changing environmental conditions increasing stress), either by increasing variance or adding one or more "over-performers" the odds of high yields were rising. The more "over-performing" provenances included the steeper the rise, as the chances of one of these provenances to be included in the subset increase with their increasing number. Given the multitude of climate factors it will be almost impossible to identify these "over-performing" provenances under any potential climatic conditions (frost, drought, heat waves etc.) within the distribution range of a species. Yet, the diversity of provenances in a stand might find a suitable answer to specific environmental conditions, without the need to know the particular performance of certain provenances under any given conditions. This increase of expected yield was only evident for the upper quantiles and maxima of expected growth; the risk of low yields was not abated. However, the low yield individuals will most probably be the ones to be outcompeted or removed in later stages of the stand's development, so a focus on the upper quantiles seems reasonable.

The same was true for the experimental data, where the strongest effect of number of provenances on expected maximum performance was revealed for the data set with the most pronounced differences between provenances (*F. sylvatica* under control conditions). This gives the hint that for species with high intra-specific differences throughout the distribution range or wide altitudinal or latitudinal ranges (Sgrò *et al.* 2011), a mixing of provenances might ensure productivity of stands under various climatic circumstances. For *P. nigra* these

high intra-specific differences have been demonstrated on a genetic level (Aguinagalde *et al.* 1997; Jagielska *et al.* 2007; Soto *et al.* 2010; Lucic *et al.* 2010), as well as in terms of general performance (Varelides *et al.* 2001; Seho *et al.* 2010; Thiel *et al.* 2012) and, of special importance, in response to abiotic conditions (Mataruga *et al.* 2010) and climatic stressors (Kreyling *et al.* 2012b). The same applies to *F. sylvatica,* for which a high intra- (Konnert 1995; Vornam *et al.* 2004) and inter-population genetic diversity (Magri *et al.* 2006) could be demonstrated. Furthermore general performance differed between provenances (vonWuehlisch *et al.* 1995) and provenances responded differently to climatic stressors such as late frost (Kreyling *et al.* 2012a) or drought (Nielsen & Jorgensen 2003; Czajkowski & Bolte 2006; Matyas *et al.* 2009; Thiel *et al.* subm.).

The ranks of expected median growth change between the treatments. For example the Hungarian provenance increases its expected median growth under maximum selection from rank to 7 under control to rank 2 under drought, whereas one of the Italian provenances (IT2) fell from rank 2 under control to rank 7 under the drought treatment. This underlines again the *insurance effect* of a high intra-specific variability (Yachi & Loreau 1999), i.e. a *portfolio* of provenances (Markowitz 1952), as the certain provenances adopt the function of high production from other provenances under changing environmental conditions.

Therefore artificial enhancement of genetic diversity and phenotypic plasticity, via "provenance-mixing", might have potential to counter the variability of climatic stressors in an uncertain future on forest ecosystem functioning. Especially when different provenances of a species are well-adapted to different climatic stressors that might occur in the future, a mixing of these provenances might be promising. For instance Kreyling *et al.* (2012a) found that Bulgarian provenances were less late frost tolerant than German provenances, and Thiel *et al*. (subm.) on the other hand found a Bulgarian provenance with a more stable growth under drought conditions compared favourable conditions than German provenances. Often an adaptation to a certain climatic environment comes at the cost of adaptation to other conditions (Hereford 2009). A mixing of such differently behaving provenance might secure productivity and functioning of stands under several climatic conditions, what might be called a *portfolio effect* in forest management (Knoke *et al.* 2005; Hanewinkel *et al.* 2011) or can be considered as a intraspecific implementation of the *insurance hypothesis* in ecology (Yachi & Loreau 1999). However, evidence suggest that a trade-off exists between high adaptedness to certain climatic or abiotic stressors and a low phenotypic plasticity in the absence of these stressors (Richter *et a.l* 2012; Thiel *et al.* subm.), i.e. highly adapted provenances might not

be able to respond to favourable conditions with an increase in yield. Therefore, a mixture of highly stress adapted provenances with local, plastically responding provenances might be one scenario to focus further research on.

Yet, a certain amount of caution is recommended here, for such a mixing might hold some problems. An introduction of genetic material (in this case non-local provenance of *F. sylvatica*) into the local gene pool might lead to the disruption of co-adapted gene complexes through new recombination and may result in outbreeding depression (Eriksson and Ekberg 2001). Still, the existence of such gene-complexes is questionable for this species as the time elapsed since the colonization of its current distribution range is quite limited (Mühltaler *et al.* 2010). Furthermore translocation of keystone species within their distribution range might influence the target locality's community structure and ecosystem processes, by changes in the genetic variation of a species (Frascaria-Lacoste & Fernández-Manjarrés 2012), yet a mixing of provenances is supposed to dampen these potentially negative effects of specific genotypes. In general the ecological risk of such a genetic translocation is considered small (Sgrò *et al.* 2011).

Besides the choice of a selection type, maximum or lottery (random), impacts the model outcome. Especially under the drought treatment provenances with high variance seem to be favoured by the maximum selection. Hence it might be important to know under which circumstances a species follows either of the paths of recruitment, before selecting provenances. However, recruitment seems to be impacted by several biotic and abiotic factors, such as herbivory or light etc. (Lamont and Witkowski 1995; Busing and Brokaw 2002), that here a diversification of a forest stands with several provenances might reduce negative impacts of these factors on recruitment.

Our sampling procedure is based on data from one growing season, in which one-year old saplings were exposed to an extreme drought, i.e. selective pressure favoured drought-resistant individuals from the beginning on. In order to draw a more realistic picture data would be required, in which provenances are exposed to favourable, local average conditions of the target locality for several years, in order to experience selection towards these conditions before being subjected to extreme climatic conditions. Generally it is supposed that, due to local adaptation processes, a trade-off in fitness exists between local and non-local environments (Sgrò *et al.* 2011). Hence it is important to know how non-local stress-adapted provenances would perform under average, non-stress conditions at the target locality. Yet, such a trade-off does not necessarily have to exist, and when it exists, is usually weak

(Hereford 2009). Besides, as mentioned above, we used individuals at very early stages in their life-cycles. On the one hand the response of these stages is very important as, successful recruitment and regeneration might be a bottle-neck under climate change conditions (Hosius *et al.* 2006), on the other hand it is questionable if seedlings-responses to climatic extremes can be transferred to mature individuals and stands. Moreover, the experimental data is derived from individually potted plants, and "provenance-mixing" is modelled in the maximum-sampling procedure. Yet, this neglects e.g. potential below-ground interactions and competition between individuals or provenances, what might alter the overall performance of a given mix of provenances.

Furthermore some model inherent problems exist. Different amounts of subsets are contained in the sets grouped by number of included provenances. Whereas the sets with intermediate number of provenance contain the most subsets, the sets with few or many provenances contain less subset (e.g. the set with just one provenance contains seven or eight subsets, according to the overall number of provenances, and the set with all provenances included contains just one subset), what impacts the comparability of results for the different groups.

Conclusion

Results from this and other studies suggest that assisted colonization of species within their distribution range, and especially the mixing of differently adapted provenances, might be a feasible means to insure forest ecosystem functioning and the provision of goods and services in the face of an uncertain future climate. Basically it seems that a *portfolio* of provenances can serve as an *insurance* against the multitude of potential adverse climate impacts.

Therefore important future climatic stressors have to be identified and different provenances have to be searched for which are well-adapted to this multitude of future extremes. The performance of these mixtures has to be tested in experiments, models and in the field, regarding stages in life-cycles (from recruitment to maturity), competitive or facilitating interactions between provenances under gradually changing climatic averages of the target locality and under climatic extreme events. Furthermore the changing interaction between climate factors and between climate and other abiotic or biotic factors (e.g. soil properties, herbivory) and their impact on these stands has to be considered. Additionally scientific focus has to be laid on the consequences of such translocations on local gene pools for future tree generations. In forestry the discussion about such an approach in climate change adaptation is

in full swing, still, for agriculture, especially in life-stock farming and pasture or for nature conservation, e.g. for the preservation of highly threatened species, the consideration of intra-specific and genetic variation might be a reasonable alternative to classic approaches, as well.

References

Aguinagalde, I., Llorente, F. & Benito, C. (1997). Relationships among five populations of European black pine (*Pinus nigra ARN.*) using morphometric and isozyme markers. *SILVAE GENETICA*, 46, 1–5.

Alsos, I.G., Ehrich, D., Thuiller, W., Eidesen, P.B., Tribsch, A. & Schönswetter, P. *et al.* (2012). Genetic consequences of climate change for northern plants. *PROCEEDINGS OF THE ROYAL SOCIETY B: BIOLOGICAL SCIENCES*. doi:10.1098/rspb.2011.2363.

Bolte, A., Ammer, C., Lof, M., Madsen, P., Nabuurs, G.-J. & Schall, P. *et al.* (2009). Adaptive forest management in central Europe: Climate change impacts, strategies and integrative concept. *SCANDINAVIAN JOURNAL OF FOREST RESEARCH*, 24, 473–482.

Bolte, A. & Degen, B. (2010). Forest adaptation to climate change - options and limitations. *LANDBAUFORSCHUNG*, 60, 111–117.

Brang, P., Bugmann, H., Bürgi, A., Mühletaler, U., Rigling A. *et al.* (2008). Klimawandel als waldbauliche Herausforderung. *SCHWEIZERISCHE ZEITSCHRIFT FÜR FORSTWESEN*, 159, 362-373.

Busing, R.T. & Brokaw, N. (2002). Tree species diversity in temperate and tropical forest gaps: the role of lottery recruitment. *FOLIA GEOBOTANICA*, 37, 33–43.

Chen, I.-C., Hill, J.K., Ohlemueller, R., Roy, D.B. & Thomas, C.D. (2011). Rapid Range Shifts of Species Associated with High Levels of Climate Warming. *SCIENCE*, 333, 1024–1026.

Czajkowski, T. & Bolte, A. (2006). Different reaction of beech (Fagus sylvatica L.) provenances from Germany and Poland to drought. *ALLGEMEINE FORST UND JAGDZEITUNG*, 177, 30–40.

Erikkson, G., Ekberg, I., (2001). An introduction to forest genetics. Genetic Center, Department of Forest Genetics, Uppsala, Sweden. 166 pp.

Frascaria-Lacoste, N. & Fernández-Manjarrés, J. (2012). Assisted Colonization of Foundation Species: Lack of Consideration of the Extended Phenotype Concept—Response to Kreyling et al. (2011). *RESTORATION ECOLOGY*, 20, 296-298.

Gessler, A., Keitel, C., Kreuzwieser, J., Matyssek, R., Seiler, W. & Rennenberg, H. (2007). Potential risks for European beech (Fagus sylvatica L.) in a changing climate. *TREES-STRUCTURE AND FUNCTION*, 21, 1–11.

Hanewinkel, M., Hummel, S. & Albrecht, A. (2011). Assessing natural hazards in forestry for risk management: a review. *EUROPEAN JOURNAL OF FOREST RESEARCH*, 130, 329–351.

Hereford, J. (2009). A Quantitative Survey of Local Adaptation and Fitness Trade-Offs. *AMERICAN NATURALIST*, 173, 579–588.

Hosius, B., Leinemann, L., Konnert, M. *et al.* (2006). Genetic aspects of forestry in Central Europe. *EUROPEAN JOURNAL OF FOREST RESEARCH*, 125, 407- 417.

Isajev V. *et al.*(2004). *EUFORGEN Technical guidelines for the genetic conservation and use of European Black pine (Pinus nigra),* Rome, Italy.

Jagielska, A., Cwalina, M. & Prus-Glowacki, W. (2007). Genetic diversity of the black pine‘s stands (Pinus the nigra) Arn. *SYLWAN*, 151, 23–31.

Jump, A.S., Marchant, R. & Penuelas, J. (2009). Environmental change and the option value of genetic diversity. *TRENDS IN PLANT SCIENCE*, 14, 51–58.

Knoke, T., Stimm, B., Ammer, C. & Moog, M. (2005). Mixed forests reconsidered: A forest economics contribution on an ecological concept. *FOREST ECOLOGY AND MANAGEMENT*, 213, 102–116.

Kolström, M., Vilen, T. & Lindner, M. (2011). Climate Change Impacts and Adaptation in European Forests. http://www.efi.int/files/attachments/publications/efi_policy_brief_6_eng_net.pdf.

Konnert, M. (1995). Investigations on the genetic variation of beech (Fagus sylvatica L) in Bavaria. *SILVAE GENETICA*, 44, 346–351.

Kreyling, J., Thiel, D., Nagy, L., Jentsch, A., Huber, G. & Konnert, M. *et al.* (2012a). Late frost sensitivity of juvenile *Fagus sylvatica* L. differs between southern Germany and Bulgaria and depends on preceding air temperature. *EUROPEAN JOURNAL OF FOREST RESEARCH*, 131, 717–725.

Kreyling, J., Bittner, T., Jaeschke, A., Jentsch, A., Steinbauer, M.J. & Thiel, D. *et al.* (2011). Assisted Colonization: A Question of Focal Units and Recipient Localities. *RESTORATION ECOLOGY*, 19, 433–440.

Kreyling, J., Wiesenberg, G., Thiel, D., Wohlfart, C., Huber, G. & Walter, J. *et al.* (2012b). Cold hardiness of *Pinus nigra Arnold* as influenced by geographic origin, warming, and extreme summer drought. *ENVIRONMENTAL AND EXPERIMENTAL BOTANY*, 78, 99–108.

Lamont, B.B. & Witkowski, E.T. (1995). A Test for lottery recruitment among 4 *Banksia* species based on their demography and biological attributes. *OECOLOGIA*, 101, 299–308.

Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *JOURNAL OF EVOLUTIONARY BIOLOGY*, 22, 1435–1446.

Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, A. & Garcia-Gonzalo, J. *et al.* (2010). Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *FOREST ECOLOGY AND MANAGEMENT*, 259, 698–709.

Lucic, A., Mladenovic-Drinic, S., Stavretovic, N., Isajev, V., Lavadinovic, V. & Rakonjac, L. *et al.* (2010). Genetic diversity of Austrian Pine (*Pinus nigra ARN.*) populations in Serbia revealedby RAPD. *ARCHIVES OF BIOLOGICAL SCIENCES*, 62, 329–336.

Magri, D., Vendramin, G.G., Comps, B., Dupanloup, I., Geburek, T. & Gomory, D. *et al.* (2006). A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. *NEW PHYTOLOGIST*, 171, 199–221.

Markowitz, H. (1952). Portfolio selection. *JOURNAL OF FINANCE* 7, 77-91.

Mataruga, M., Haase, D.L. & Isajev, V. (2010). Dynamics of seed imbibition and germination of Austrian pine (*Pinus nigra Arnold*) from extreme habitat conditions within five Balkan provenances. *NEW FORESTS*, 40, 229–242.

Matyas, C., Bozic, G., Goemoery, D., Ivankovic, M. & Rasztovits, E. (2009). Juvenile growth response of European beech (*Fagus sylvatica L.*) to sudden change of climatic environment in SE European trials. *IFOREST-BIOGEOSCIENCES AND FORESTRY*, 2, 213–220.

Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J. & Mathesius, U. *et al.* (2010). Plant phenotypic plasticity in a changing climate. *TRENDS IN PLANT SCIENCE*, 15, 684–692.

Nielsen, C.N. & Jorgensen, F.V. (2003). Phenology and diameter increment in seedlings of European beech (*Fagus sylvatica L.*) as affected by different soil water contents: variation between and within provenances. *FOREST ECOLOGY AND MANAGEMENT*, 174, 233–249.

Ohlemuller, R., Gritti, E.S., Sykes, M.T. & Thomas, C.D. (2006). Quantifying components of risk for European woody species under climate change. *GLOBAL CHANGE BIOLOGY*, 12, 1788–1799.

Pedlar, J.H., McKenney, D.W., Beaulieu, J., Colombo, S.J., McLachlan, J.S. & O'Neill, G.A. (2011). The implementation of assisted migration in Canadian forests. *FORESTRY CHRONICLE*, 87, 766–777.

Richter, S., Kipfer, T., Wohlgemuth, T., Calderón Guerrero, C., Ghazoul, J. & Moser, B. Phenotypic plasticity facilitates resistance to climate change in a highly variable environment. *OECOLOGIA*, 1–11.

Rose, L., Leuschner, C., Koeckemann, B. & Buschmann, H. (2009). Are marginal beech (Fagus sylvatica L.) provenances a source for drought tolerant ecotypes? *EUROPEAN JOURNAL OF FOREST RESEARCH*, 128, 335–343.

Seho, M., Kohnle, U., Albrecht, A. & Lenk, E. (2010). Growth analyses of four provenances of European Black Pine (*Pinus nigra*) growing on dry sites in southwest Germany (Baden-Wuerttemberg). *ALLGEMEINE FORST UND JAGDZEITUNG*, 181, 104–116.

Sgrò, C.M., Lowe, A.J. & Hoffmann, A.A. (2011). Building evolutionary resilience for conserving biodiversity under climate change. *EVOLUTIONARY APPLICATIONS*, 4, 326-337.

Soto, A., Robledo-Arnuncio, J.J., Gonzalez-Martinez, S.C., Smouse, P.E. & Alia, R. (2010). Climatic niche and neutral genetic diversity of the six Iberian pine species: a retrospective and prospective view. *MOLECULAR ECOLOGY*, 19, 1396–1409.

Thiel, D., Kreyling, J., Backhaus, S., Beierkuhnlein, C., Buhk, C. *et al.* (in press). Different reactions of central and marginal provenances of Fagus sylvatica to experimental drought. *EUROPEAN JOURNAL OF FOREST RESEARCH.*

Thiel, D., Nagy, L., Beierkuhnlein, C., Huber, G., Jentsch, A. & Konnert, M. *et al.* (2012). Uniform drought and warming responses in *Pinus nigra* provenances despite specific overall performances. *FOREST ECOLOGY AND MANAGEMENT*, 270, 200–208.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J. & Collingham, Y.C. *et al.* (2004). Extinction risk from climate change. *NATURE*, 427, 145–148.

Varelides, C., Brofas, G. & Varelides, Y. (2001). Provenance variation in *Pinus nigra* at three sites in Northern Greece. *ANNALS OF FOREST SCIENCE*, 58, 893–900.

vonWuehlisch, G., Krusche, D. & Muhs, H.J. (1995). Variation in temperature sum requirement for flushing of beech provenances. *SILVAE GENETICA*, 44, 343–346.

Vornam, B., Decarli, N. & Gailing, O. (2004). Spatial distribution of genetic variation in a natural beech stand (*Fagus sylvatica L.*) based on microsatellite markers. *CONSERVATION GENETICS*, 5, 561–570.

Wortemann, R., Herbette, S., Barigah, T.S., Fumanal, B., Alia, R. & Ducousso, A. *et al.* (2011). Genotypic variability and phenotypic plasticity of cavitation resistance in Fagus sylvatica L. across Europe. *TREE PHYSIOLOGY*, 31, 1175–1182.

Yachi, S. & Loreau, M. (1999). Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA*, 96, 1463–1468.

Yousefpour, R. & Hanewinkel, M. (2009). Modelling of forest conversion planning with an adaptive simulation-optimization approach and simultaneous consideration of the values of timber, carbon and biodiversity. *ECOLOGICAL ECONOMICS*, 68, 1711–1722.

Zhu, K., Woodall, C.W. & Clark, J.S. (2012). Failure to migrate: lack of tree range expansion in response to climate change. *GLOBAL CHANGE BIOLOGY*, 18, 1042-1052.

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8. Appendix

Further publications:

- Zanchi, G., **Thiel, D**., Green, T., Lindner, M. (2007). Forest Area Change and Afforestation in Europe. Critical Analysis of Available Data and the relevance for International Environmental policy. EFI Technical Reports, EFI, Joenuu, Finnland.

- **Thiel, D.,** Kreyling, J., Beierkuhnlein, C., (2011). Anpassungsoptionen für unsere Wälder. Innerartliche Unterschiede bei Bäumen bezüglich der Reaktion auf den Klimawandel, SPEKTRUM Universität Bayreuth 7 (1), 74-77.

- Kreyling, J., Huber, G. Jentsch, A., Konnert, M., Nagy, L., **Thiel, D.,** Wellstein, C, Beierkuhnlein, C. (2011). Innerartliche Plastizität und lokale Anpassung bei Waldbäumen. LWF Aktuell 25, 12-14.

9. Erklärung

Hiermit erkäre ich, dass ich die Arbeit selbständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe.

Ferner erkläre ich, dass ich anderweitig mit oder ohne Erfolg nicht versucht habe, diese Dissertation einzureichen. Ich habe keine gleichartige Doktorprüfung an einer anderen Hochschule endgültig nicht bestanden.

Teisendorf, 26.11.2013